Stephen J. Withrow
David M. Vail

Withrow & MacEwen's

SMALL ANIMAL
CLINICAL ONCOLOGY

Fourth Edition
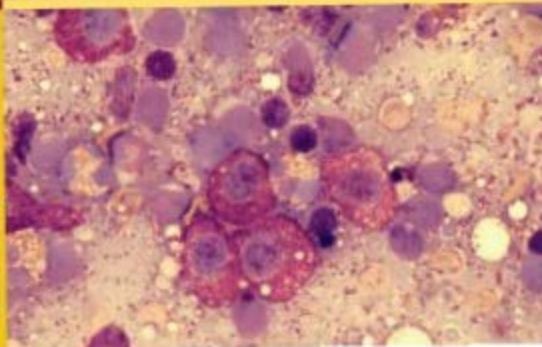
WITHROW AND MACEWEN'S SMALL ANIMAL
CLINICAL ONCOLOGY

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PREFACE

Several important milestones in veterinary oncology have occurred between the publication of the third edition of the textbook in 2001 and the current edition. One in particular represents a significant loss to the community and indeed this publication; the passing of E. Gregory MacEwen. Greg was a pioneering leader in the field of veterinary oncology and was responsible for the development of several novel and innovative cancer therapies for both companion animals and people. Invariably those of us that were touched by him professionally also developed a deep and heart-felt personal relationship with this kind and gentle man. His untimely passing left a tremendous void; however, he continues to serve as an inspiration to those in the field, and the work that he initiated continues to advance the art and science of oncology, in no small part due to the scores of veterinary students, residents, and post graduate students Greg educated and inspired.

Many positive milestones have also occurred, not the least of which has been the characterization and publication of the canine genome by a group from MIT (Nature 438:803-819, 2005) that allows us to investigate the genetic basis of cancer within and between species. This milestone was followed shortly by the development of two consortia centered at the Comparative Oncology Program at the National Cancer Institute (NCI; USA); the Canine Comparative Oncology Genomics Consortium (CCOGC) and the Comparative Oncology Trials Consortium (COTC). These consortia, developed largely through significant effort put forth by the Comparative Oncology Program, have brought together veterinary and medical oncologists, pathologists, surgeons, geneticists, and molecular and cellular biologists with the goal of facilitating strategic partnerships and collaborations across a diversity of disciplines, focusing on the problem of cancer in veterinary and human patients. The CCOGC maintains a biospecimen repository centered at the NCI that is available for comparative analysis. The COTC central mission is the development and delivery of novel cancer treatment strategies for cancer patients by performance of well controlled and randomized companion animal trials in collaboration with NCI investigators, academic institutions, and the pharmaceutical industry. These trials are implemented through the collective caseloads of the consortium membership with trial oversight and data management provided by the Comparative Oncology Program. These milestones represent a "coming of age" for veterinary oncology and the application of scientific method and clinical investigation in a rigorous and controlled atmosphere. As we go to press with this text, the Morris Animal Foundation is preparing to launch a 22 million dollar campaign in support of the COTC and the CCOGC. This endowment will initiate and support data collection and prevention studies. We hope the fifth edition will lead off with an announcement of MAF's success on behalf of the canine cancer patients.

Additionally, important advances in our understanding of the molecular aberrations occurring in the development and progression of cancer have led to the characterization of several important cancer pathways and identified new targets for cancer prevention and therapy. This fourth edition is replete with discussion on the molecular basis of cancer development and cancer therapy.

During the planning of this edition, we made the decision to produce a book that was clinically relevant for the general practitioner and veterinary medical student as well as being a state-of-the-art text with an emphasis on the molecular basis, biologic behavior, and treatment of cancer in small animals. The fourth edition follows a similar format as the first three editions. We begin with introductory chapters on the biology and pathogenesis of cancer and follow with a section of chapters on the diagnosis and staging of the cancer patient. A general section covering the varied therapeutic modalities available to the cancer patient is then followed by a section outlining specific malignancies encountered in small animal veterinary patients; their etiology, presentation, diagnosis, therapy, and prognosis.

All chapters have been extensively reworked and revised, and the addition of color to the current edition has an immediate impact on the aesthetics of the overall work. Care has been taken to include therapy protocols that have been evaluated with sufficient case material to allow a determination of efficacy. Noteworthy additions to the current edition include chapters on the Etiology of Cancer, Molecular Diagnostics, Molecular Targeted Therapy, and Supportive Cancer Care. We are delighted at the exceptional quality of the chapters our invited authors produced. We are particularly pleased with our publisher, Elsevier. We especially want to thank Tony Winkel, our editor, and Shelly Stringer, Developmental Editor, of the Elsevier staff. We also want to thank Rich Barber, production Manager, and Jennifer Hong, editorial assistant, for their detail in editing the typed manuscript. Special thanks are extended to Carol Horner at the Animal Cancer Center at Colorado State University for clerical and transcription services.

It is our intent to provide the reader with the most current information in clinical oncology and a better understanding of cancer biology, diagnostic approaches, therapeutic interventions, and prognostic and predictive variables. Cancer is not always curable, but in most cases the patient can be helped to an improved quality and quantity of life.

Stephen J. Withrow, D.V.M.
David M. Vail, D.V.M.

DEDICATION

We dedicate this edition to these fine men, each of whom pioneered their
particular branch of veterinary oncology:

Dr. E. Gregory MacEwen
1943-2001
The father of veterinary medical oncology, Greg was personally responsible for
educating and inspiring the current generation of medical oncologists, both as clinicians
and clinician scientists.

Dr. Robert S. Brodey
1927-1979
The father of veterinary surgical oncology, Bob will be remembered for his tireless
effort to advance the field of oncology, to teach principles of surgery, and, most importantly,
to preserve nature.

Dr. Edward L. Gillette
The father of veterinary radiation oncology, Ed is a leader in comparative oncology. His vision
and leadership have created a new and contemporary breed of oncologists in all disciplines.

Why Worry About Cancer in Pets?

Stephen J. Withrow

Why should veterinarians be concerned about cancer in pet animals? Unfortunately, the prevalence of cancer in pet animals continues to rise. Prevalence is an increased number of diagnosed cancer cases per year without documenting the number versus the population at risk (incidence). This prevalence is increasing for a variety of reasons but is at least in part related to animals living to increasingly older ages. Since cancer is generally a disease of the older animal, the price they pay for living longer is an increased likelihood of developing cancer. The greater life span is a result of better nutrition, vaccinations (preventing many previously fatal contagious diseases), better preventive and therapeutic medical practices, leash laws, and possibly a deeper devotion (human-animal bond) to pet animals within the last 10 to 20 years. With this increasing prevalence, veterinarians will be called upon more frequently to diagnose and manage the pet with cancer.

Cancer is a major cause of pet animal death (Chapter 4).[1-4] This statement is supported by a study that determined the cause of death in a series of more than 2000 necropsy cases. In that study, 45% of dogs that lived to 10 years or older died of cancer.[3] With no age adjustment, 23% of patients presented for necropsy died of cancer. Of the more than 74 million household dogs and 90 million cats in the United States (AVMA), at least 4 million dogs and 4 million cats may develop cancer each year.[5] In a 1998 Mark Morris Foundation Animal Health Survey, more than 2000 respondents stated that cancer was the leading cause of disease-related death in both dogs (47%) and cats (32%).[2] Another Morris survey done in 2005 revealed that cancer was by far the largest health concern among pet owners (41%), with heart disease the number two concern at 7%. Regardless of the exact numbers, cancer is one of the leading killers of pet animals and at the forefront of our clients' thoughts as it relates to the health of their companions.

Breakthroughs in treatment of human cancer have received a great deal of exposure through the news media, popular press, and website information services. Although progress is slow, it does expose pet owners to what can be done and promotes an atmosphere of optimism. With this increased and optimistic media coverage, pet owners are becoming more knowledgeable and demanding in seeking care for the animal with cancer. The veterinary profession needs to be prepared for these demands.

More open acknowledgment of the human-animal bond has elevated the importance of pet animals to the level of human beings in many owners' eyes. Some owners consider their pet more important than any human contact (Chapter 16, Section D).[5] Proper health care of pet animals will be of increasing importance to many owners.

Cancer is a common and serious disease for human beings. Many owners have had or will have a personal experience with cancer in themselves, a family member, or a close friend. Realizing the importance of pets to owners, it must be realized that owners value the veterinarian's ability to care as much as his or her ability to cure. Keeping this in mind, the veterinarian should approach the pet with cancer in a positive, compassionate, and knowledgeable manner. Frequently the veterinary profession has taken a negative approach ("test and slaughter") to cancer. This attitude will not only be a detriment to the pet but may also negatively reinforce unfounded fears in the owner about the disease in humans. We owe it to our pet animal patients and their owners to be well informed and up-to-date on current treatment methods to prevent imparting unnecessary feelings of hopelessness.

Pet animals with spontaneously developing cancer provide an excellent opportunity to study many aspects of cancer from etiology to treatment. The National Cancer Institute has recently approved the Comparative Oncology Trials Consortia (COTC), which is a collection of 14 veterinary schools united to perform clinical trials on dogs and cats with cancer. Access to new drugs and biologics of interest to the Children's Oncology Group, National Cancer Institute, the Food and Drug Administration, and the pharmaceutical industry will speed clinical applications for both species. Providing these studies are done in a humane fashion, they may unlock clues to improving the outlook for this disease in both animals and humans.[6-10] Some of the aspects of

pet animal cancer that enable attractive comparative models include the following:

1. Pet dogs and cats are genetically outbred animals (like humans) as opposed to some strains of rats, mice, and other experimental animals.
2. The cancers seen in practice are spontaneously developing as opposed to experimental carcinogen-induced cancers. Spontaneous cancers may behave in a significantly different fashion than induced or transplanted cancers.
3. Pets share the same environment as their owners and may serve as epidemiologic or etiologic sentinels for the changing patterns of cancer development seen in humans.
4. Pets have a higher incidence of some cancers (e.g., osteosarcoma, non-Hodgkin's lymphoma, and others) than humans, allowing more cases to be studied.[11,12]
5. Most animal cancers will progress at a more rapid rate than will the human counterpart. This permits more rapid determination of end points such as time to metastasis, local recurrence, and survival.
6. Because fewer established "gold standard" treatments exist in veterinary medicine compared to human medicine, it is easier and morally acceptable to attempt new forms of therapy (especially single-agent trials) on an untreated cancer rather than wait to initiate new treatments until all "known" treatments have failed, as is common in the human condition. Unfortunately, this latitude in clinical trials can be abused to allow diverse and unethical treatments to be attempted as well. We have an obligation to not deny our patients known effective treatment while at the same time planning well-designed prospective clinical trials of newer treatment methods.

   Investigations of new treatment methods are becoming more and more difficult to perform on normal laboratory animals because of the animal rights movement. This will make spontaneously occurring pet animal cancer a more attractive and morally acceptable research tool in the future but should not imply that poorly conceived and executed "research" is permissible on any animal.
7. Pet animal cancers are more akin to human cancers than are rodent tumors in terms of patient size and cell kinetics. Dogs and cats also share similar characteristics of physiology and metabolism for most organ systems and drugs. Such correspondence allows better comparison of treatment modalities such as surgery, radiation, and chemotherapy between animals and humans to be made.
8. Dogs and cats have intact immune systems as opposed to certain rodent systems or *in vitro* cancer studies, which allows immunologic assays and treatment approaches to be explored.
9. Animal trials are generally more economical to run than human trials.
10. Animals live long enough to determine the potential late effects of treatment.
11. Regional referral centers exist to concentrate case accrual and facilitate clinical trials.
12. Owners are often willing to allow a necropsy, which is a crucial end point for not only tumor control but toxicity.
13. Dogs and cats are large enough for various imaging studies as well as surgical intervention.
14. Radiation field sizes and chemotherapy doses and toxicity resemble those in humans.
15. The recent elucidation of the canine genome and its resemblance and relevance to the human genome opens unique and unparalleled opportunity to study comparative oncology from a genetic perspective.[13]

Owners who seek treatment for their pet animals with cancer are a devoted and compassionate subset of the population. Working with these owners can be a very satisfying aspect of a sometimes frustrating specialty. These owners are almost always satisfied with an honest and aggressive attempt to cure or palliate the disease of their pet, making the experience rewarding for the veterinarian, for the owner, and, most important, for the pet.

Oncology also offers the inquisitive veterinarian a complex and challenging area for both clinical and bench research. The challenges and accomplishments in oncology have been very impressive. Oncology offers unlimited opportunity for the pursuit of knowledge for the benefit of animals and humankind. "Cancer, unlike politics and religion, is not a topic of controversy. No one is for it. Cancer is not another word for death. Neither is it a single disease for which there is one cure. Instead, it takes many forms, and each form responds differently to treatment."[14]

Clinical and comparative oncology is a rapidly growing field of study.[15] More training programs are being developed each year that will allow a wider distribution of experienced veterinarians into practice, research, industry, government, and the academic setting. Through study and treatment of pet animal cancer, the veterinarian can hopefully impact both the pets afflicted with this disease and its development and treatment in humans.

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1

The Etiology of Cancer

SECTION A
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CANCER IS A GENETIC DISEASE

The evidence for the genetic origin of cancer is now irrefutable.[1,2] The convergence of various observations in the realms of pathology, radiation biology, DNA structure, microbiology, epidemiology, and cytogenetics in the 1950s, 1960s, and 1970s paved the way for the identification of a multitude of cancer-related genes. Nowell and Rowley documented and characterized recurrent cytogenetic abnormalities and their relationship to cancer, Knudson described the existence of heritable cancer syndromes,[3] and Leder showed that inappropriate expression of a single oncogene was sufficient to produce cancer in mice.[4] Since then, several hundred genes that can act as oncogenes or tumor suppressor genes have been identified. Lists of these genes can be found in many contemporary reviews,[1] and we will not repeat them here. Rather, we will focus on new information that will assist the reader in understanding the genetic basis of cancer and how the interactions between genes and environment impact the origin, progression, and response to therapy of most tumors (Box 1-1).

Genetics of Initiation, Promotion, and Progression

The "initiation, promotion, and progression" (IPP) model[5,6] is a useful starting point to define the genetic basis of cancer. Unlike diseases due to single-gene defects, cancer is a complex, multigenic disease, and this model is among the first to recognize a sequential progression of mutations that can account for cancer. In the IPP model, a genetic mutation endows a somatic cell with limitless replicative potential or another growth or survival advantage from other cells in its environment (initiation).

Alone, this mutation is not sufficient to give rise to a tumor, as the cell remains constrained by environmental factors. A second mutation (or series of mutations) further adds to the cell's ability to out-compete its neighbors in this environment, leading to its potential expansion into a recognizable tumor mass (promotion). Finally, a third series of mutations reinforces the cell's malignant potential (invasion, tissue destruction, and metastasis) that lead to clinical disease (progression).
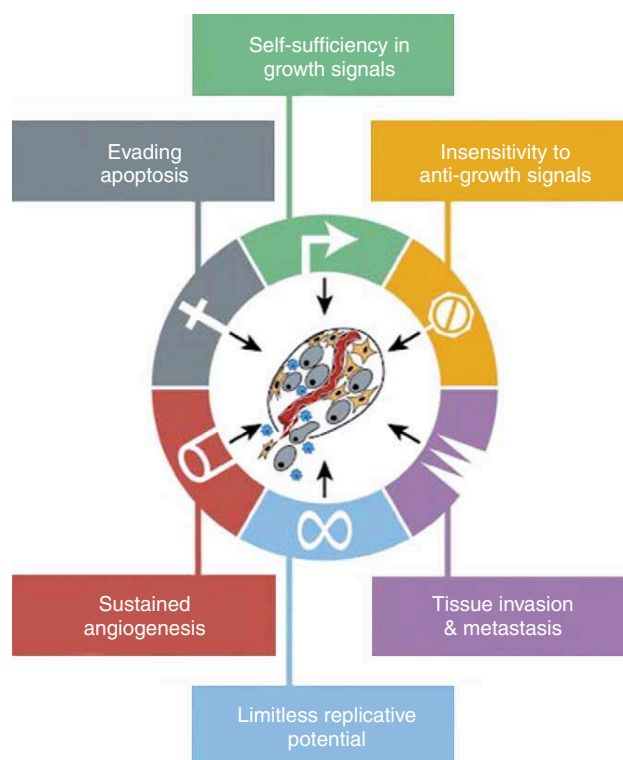
This model illustrates several important points in our current understanding of cancer, but as importantly, it poses several questions for which we do not yet have a definitive answer. First, one must note that we have chosen to avoid "naming names" with respect to genes that are associated with these putative steps to carcinogenesis, and that is because no single gene is universally responsible for transformation. Rather, many genes contribute to (rather than "cause") the origin and progression of cancer.[1] Second, this provides a basis to estimate the minimum number of mutations that are required before cancer becomes clinically evident. Hanahan and Weinberg[7] described in detail the sequential process of mutations that a cell must acquire in this process. It is estimated that five or six mutations are the minimum number that can provide a cell and its progeny with each of six hallmarks of cancer (Figure 1-1). These include (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) the ability to evade apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) the capacity to invade tissues and metastasize. On occasion, a single gene may confer more than one such property that contributes to the origin or progression of a tumor, but more often, multiple mutations are needed to achieve each of these "milestones." In addition, each of these steps is regulated by multiple interactive biochemical pathways; thus, mutations of different genes along a pathway can result in equivalent phenotypes. Hahn and Weinberg eloquently described this as a "molecular circuitry" of cancer[8] (see www.nature.com/nrc/poster/subpathways/index.html), where mutation of a gene is not isolated but rather can affect multiple biological processes along these interactive

Evidence and Implications for the Genetic Basis of Cancer

- The genetic basis of cancer is beyond question.
- Although abnormalities in a small number of genes appear to occur disproportionately in many or most cancers, a much larger number of genes have been implicated on the origin and progression of tumors in humans and animals.
- Sequential mutations of at least five genes are facilitated by (or work in synergy with) genomic instability to establish a self-renewing population of cells whose progeny undergo natural selection.
- Tumor populations may arise stochastically but more likely originate from cancer stem cells.
- The expansion of the tumor populations expansion leads to clinical disease; metastasis and drug resistance are usually the cause of death.
- Improvements in prevention and therapy will require a clear and thorough understanding of the genetic basis of cancer to devise strategies that inhibit or reverse the events associated with transformation and progression.

Figure 1-1

Acquired capabilities of cancer. Most if not all cancers acquire the same set of functional capabilities during their development, albeit through multiple mechanistic strategies. *(Reproduced with permission from D. Hanahan and R. Weinberg, "The Hallmarks of Cancer," Cell 100:57–70, 2000. ©Elsevier, UK.)*

networks. Conceptually, this helps us understand how mutations of different genes can lead to similar cancer phenotype and outcome, and conversely why mutations of the same gene can result in different cancers with distinct biological behaviors.

Our previous reference to a "selective growth advantage" that is reminiscent of Darwinian selection, and which is recognized in each of the reviews we have cited, is not accidental. The clonal evolution theory[9,10] addresses the significance of sequential genetic changes providing growth and survival advantages, but to this we must add the fact that, in addition to these self-sufficient events that influence growth and survival, tumor cells must also evade "predators" (e.g., inflammation and the immune system[11,12]). In essence, the interaction of the tumor with its microenvironment, and ultimately with the host, is in fact subject to Darwinian laws of evolution, albeit in an accelerated time scale.[13] This is evident in the ability of tumors to modulate stromal cells to support their own growth by providing a suitable matrix and an abundance of nutrients while keeping antitumor responses at bay. Thus, there is a bidirectional influence on stromal cells, where normal stroma prevents tumor growth, but the tumor produces factors that influence the generation of a supportive stromal environment, and some of these same factors (such as transforming growth factor β and cytokines that activate the Stat-3 protein) prevent the host from mounting an effective antitumor response.[14–18]

In some cases, it is possible that progeny from the tumor cells themselves "evolve" to form stromal or vascular structures that support tumor growth.[19]

As is true for other selective environments, tumors that outgrow the capability of their immediate surroundings to support their growth will seek to become established in other favorable locations. This process of metastasis, which usually kills the host, was deconstructed by Fidler in his adaptation of Padget's "seed and soil" hypothesis into a similar model to that proposed previously.[20] He suggested that there is a stepwise acquisition of assets that enables cells (seeds) to leave the primary tumor site, travel through blood or lymph, invade stroma in favorable locations (soil), and thus become reestablished at distant sites (Figure 1-2). Work by Muschel and her colleagues[21–23] allows us to update this model by understanding that most tumors inherently possess the ability to dislodge cells that travel to distant sites, and it may be the ability of such cells to survive in capillary beds at such sites that is probably most important in the
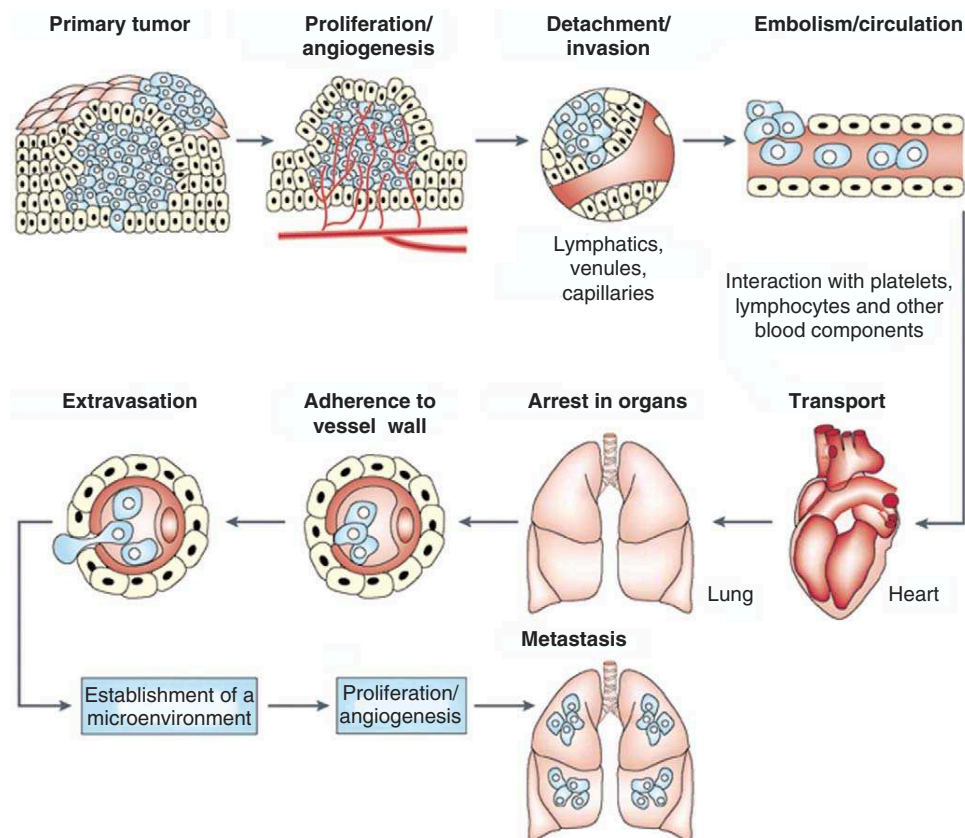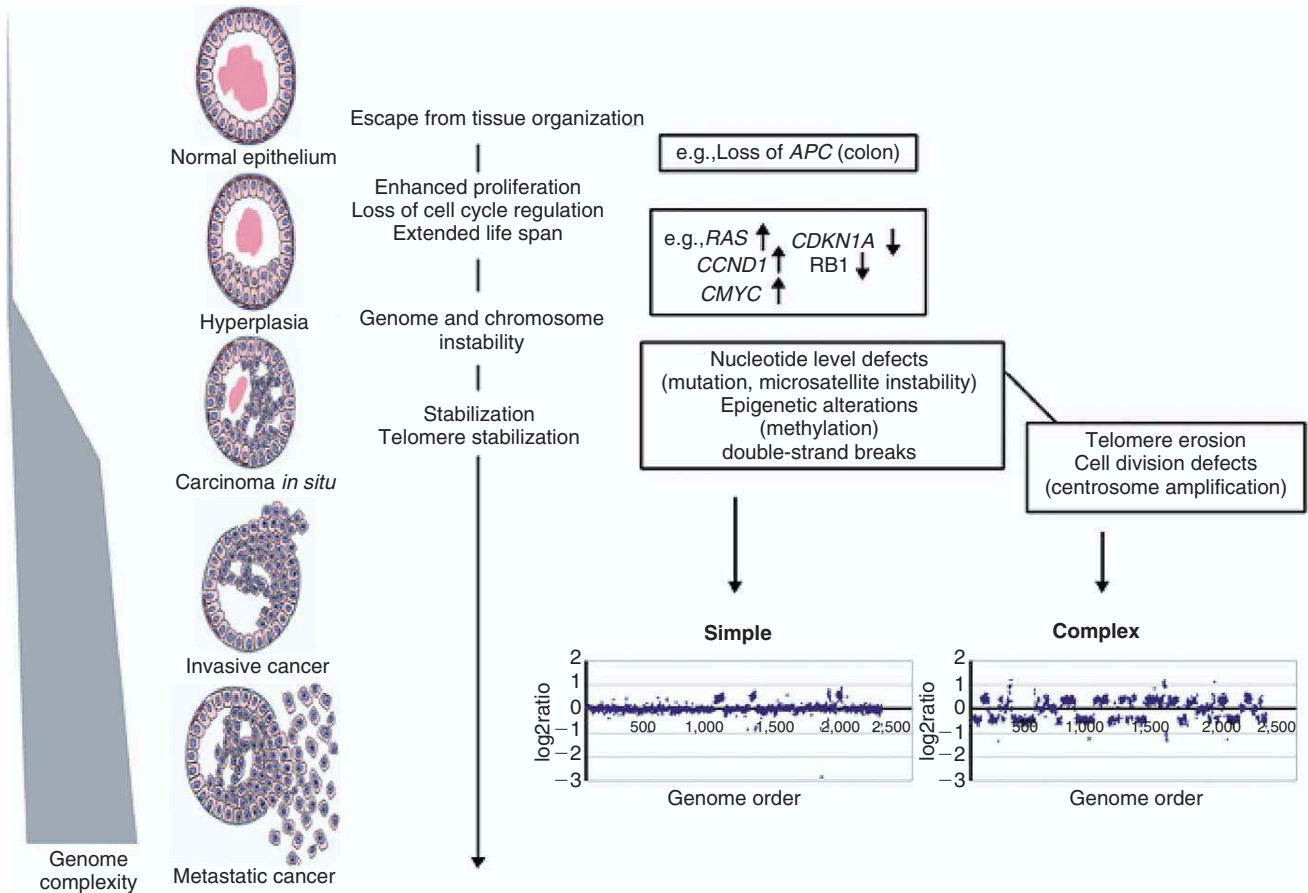
Figure 1-2

The main steps in the formation of a metastasis. Cellular transformation and tumor growth. Growth of neoplastic cells must be progressive, with nutrients for the expanding tumor mass initially supplied by simple diffusion. Extensive vascularization must occur if a tumor mass is to exceed 1 to 2 mm in diameter. The synthesis and secretion of angiogenic factors establish a capillary network from the surrounding host tissue. Local invasion of the host stroma by some tumor cells occurs by several parallel mechanisms. Thin-walled venules, such as lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most common route for tumor cell entry into the circulation. Detachment and embolization of single tumor cells or aggregates occurs next, most circulating tumor cells being rapidly destroyed. After the tumor cells have survived the circulation, they become trapped in the capillary beds of distant organs by adhering either to capillary endothelial cells or to subendothelial basement membrane that might be exposed. Extravasation occurs next, probably by mechanisms similar to those that operate during invasion. Proliferation within the organ parenchyma completes the metastatic process. To continue growing, the micrometastasis must develop a vascular network and evade destruction by host defenses. The cells can then invade blood vessels, enter the circulation and produce additional metastases. *(Reproduced with permission from I. J. Fidler, "The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited,"* Nature Reviews Cancer 3:1–6, 2003. *©Nature Publishing Group.)*

metastatic process. This also explains why metastases occur most frequently in organs that have extensive capillary networks (e.g., lung, liver). Extravasation occurs by simple exit through capillary fenestrations or, once the metastatic tumor becomes large enough to displace (or kill) endothelial lining cells, ready access to new stromal beds is achieved.

Genetic Instability in Carcinogenesis

The stepwise model of clonal evolution is satisfying because it can be correlated with discrete pathological changes in tumor progression, especially for epithelial tumors where such progression can be seen in lesions that go through stages of hyperplasia, atypical hyperplasia (dysplasia), adenoma, carcinoma *in situ*, invasive

Escape from tissue organization

e.g.,Loss of *APC* (colon)

Enhanced proliferation
Loss of cell cycle regulation
Extended life span

e.g.,*RAS* ↑ *CDKN1A* ↓
CCND1 ↑ RB1 ↓
CMYC ↑

Genome and chromosome
instability

Nucleotide level defects
(mutation, microsatellite instability)
Epigenetic alterations
(methylation)
double-strand breaks

Stabilization
Telomere stabilization

Telomere erosion
Cell division defects
(centrosome amplification)

Normal epithelium

Hyperplasia

Carcinoma *in situ*

Invasive cancer

Metastatic cancer

Genome
complexity

Simple

Complex

Genome order

Genome order

log2ratio

log2ratio

Figure 1-3

Schematic illustration of chromosomal evolution in human solid tumor progression. The stages of progression are arranged with the earlier lesions at the top. Cells may begin to proliferate excessively owing to loss of tissue architecture, loss of checkpoints, and other factors. In general, relatively few aberrations occur before the development of *in situ* cancer. As indicated, a sharp increase in genome complexity (the number of independent chromosomal aberrations) in many (but not all) tumors coincides with the development of *in situ* disease. The types and range in aberration number varies markedly between tumors, probably owing to the specific failures in checkpoint or damage surveillance that are present, as illustrated by the whole genome array CGH profiles of HCT116, a mismatch repair-defective cell line, and T47D, a mismatch repair-proficient cell line. The copy number profiles of HCT116 and T47D are labeled as "simple" and "complex," respectively, to distinguish between tumor genomes with few or many copy number changes. The spectrum of aberrations in *in situ* lesions is similar to those found in more advanced malignancies. Thus, an early increase in chromosome aberration composition is followed by more modest chromosomal evolution. *(Reproduced with permission from D. G. Albertson et al., "Chromosome aberrations in solid tumors,"* Nature Genetics 34:369–376, 2003. ©Nature Publishing Group.)

carcinoma, and metastatic carcinoma (Figure 1-3). However, analysis of tumor genomes even in early stages usually shows aneuploidy (abnormal DNA copy number) as well as chaotic changes indicative of multiple numerical and structural DNA abnormalities. Boveri first noticed similar abnormalities in the early 1900s in studies of sea urchin cells, which led him to formulate the "aneuploidy theory" of cancer.[3,24,25] We know now that aneuploidy is especially evident in solid tumors; based on this, Loeb proposed the existence of a "mutator phenotype,"

where cells are predisposed to undergo multiple mutations, some of which inevitably lead to cancer.[26] Some tenets of his hypothesis appear to be correct, although perhaps in different circumstances than Loeb originally envisioned, as they might relate to increased activity of polymerases with low fidelity under conditions where the rate of DNA damage (and consequently mutations) is higher than the expected background from normal DNA replication (for example, in lung epithelial cells from heavy smokers). However, direct measurements of

mutation rates of sporadic tumors are much lower than those predicted if a "mutator phenotype" was operative in these tumors.[25]

Nevertheless, genetic instability is a hallmark of tumors, and while it can be partly explained by increased errors in DNA replication and chromosomal segregation in cells that are rapidly dividing, other mechanisms are clearly operative, involving telomeres and telomerase, as well as mechanisms of DNA replication and repair and chromosomal segregation.[13,24,25,27–29] Although many of these changes are not "recurrent" and appear to be random products of instability, some may in fact contribute to proliferative crisis.[30] It is possible that the initiation events for many tumors occur early in life during highly proliferative stages of tissue growth and remodeling (e.g., prior to closure of the growth plates in bone cancer), but they become evident late in life when a last series of mutations allows the transformed cell to reach a crisis stage. Intriguingly, hematopoietic tumors seem to avoid the chaotic chromosomal instability associated with solid tumors. We do not fully understand the reasons for this, although it may be partly due to intrinsic protective mechanisms associated with the proliferative rate of bone marrow precursor cells.

Epigenetic Events and Cancer

Another observation is that the events leading to cancer need not necessarily be caused by mutational events, but instead can be caused by epigenetic changes. Epigenetic events are those that can alter phenotype without changing the genotype. Two well-characterized epigenetic mechanisms regulate gene expression. Gene silencing can occur by methylation of CpG residues in promoter regions, as well as by histone deacetylation. Both of these events interfere with the transcriptional machinery and repress gene expression. The effects of global changes in methylation or deacetylation (for example, by inactivation of DNA methylases or histone deacetylases) remain incompletely understood, but silencing of specific genes by methylation is implicated in numerous cancers of humans and animals.[2,31–33] One important observation is that most (or all) genes that are subject to silencing by methylation in specific cancers (e.g., *Ink4a* in T cell leukemia) are commonly inactivated by mutation or deletion in other cancers (e.g., *Ink4a* in melanoma).

As is true for mutations (as discussed later), gene regulation by epigenetic methylation can occur sporadically or it can be heritable. Silencing of some tumor suppressor genes in sporadic cancers occurs more frequently by epigenetic methylation than by mutation or deletion. These different mechanisms of gene silencing are not equivalent, as they each result in specific tumor phenotypes. For example, data from our laboratories indicate that loss of canine chromosome 11 (CFA 11) with resultant deletion of the *INK4* tumor suppressor locus, and methylation of the *Ink4a* gene, are each associated with morphologically distinct types of T cell lymphoma that have different clinical presentation and prognosis.

Genomic imprinting presents a unique example where heritable epigenetic changes influence cancer predisposition. Genomic imprinting refers to a pattern of gene expression that is determined by the parental origin of the gene; in other words, unlike most genes where both parental alleles are expressed, only one allele (specifically derived from the mother or from the father, depending on the gene) of an imprinted gene is expressed and the other one is permanently repressed. Epigenetic changes in Wilms' tumor and in heritable colon cancer (among others) alter the expression of the imprinted allele, leading to loss of imprinting (LOI) that causes *overexpression* of the insulin growth factor-2 (*Igf2*) gene.[2,34]

Gene and Environment Interactions in Cancer

The genetic predisposition to cancer is clearly modulated by heritable factors, as is illustrated by the existence of well-defined heritable cancer syndromes.[35] Even though they account for less than 5% of all human cancers, studies of families with these syndromes provided many of the initial clues to understand the genetic basis of sporadic cancers. In most cases, these syndromes show dominant patterns of inheritance and have high penetrance, although the mechanism of disease is not due to inheritance of a dominant allele but rather is generally due to inheritance of a mutant (inactive) allele followed by inactivation of the second allele through a process called "loss of heterozygosity" (LOH). In some cases, such as bone tumors in kindreds that carry mutations in the retinoblastoma (RB) tumor suppressor gene, imprinting of a mutant allele can contribute to the process of tumorigenesis.[36] A curious observation worth noting is that different mutations in a single gene predispose individuals to distinct cancer syndromes, while independent, single mutations of different genes can result in virtually the same disease, or at least diseases with indistinguishable phenotypes.[35] This is less surprising when we consider the fact that commonly affected genes are multifunctional and are parts of complex interactive networks or circuits,[8] so any single mutation only alters gene function along one biochemical pathway, leaving its interactions with other pathways intact. Another intriguing observation is that mutations that contribute to most sporadic cancers are restricted to a small subset of genes,[25] many of which also are associated with heritable cancer syndromes.

At least one heritable cancer syndrome (renal carcinoma and nodular dermatofibrosis or RCND of German shepherd dogs) has been described in dogs.[37] The heritable factor (or *RCND* gene) for this syndrome maps to CFA 5, specifically to the folliculin gene, which has been

described as the heritable factor for the corresponding human disease (Birt-Hogg-Dube syndrome). It is probable that other comparable syndromes to those that are described in humans will be identified in companion and laboratory animals, but it is unlikely these will account for more than 5% to 10% of all cancer cases. Yet even with the estimated lifetime cancer risk (for humans and for dogs) of approximately 1 in 3, it is incorrect to surmise that most cancers (>90%) are due solely to environmental causes. Heritable influences affect susceptibility, but they are difficult to identify in heterogeneous outbred backgrounds such as those that occur in most human families or mixed-breed dogs. On the other hand, highly inbred human populations and families, specific ethnic groups, or purebred dogs may allow for identification of factors that influence both cancer risk and phenotype.[38-40] Outside of these populations, the existence of heritable factors that are modulated by environmental influence will be laborious to define. For example, more than 3000 matched cases and controls would be needed to unequivocally identify the multiplicative interaction among two or more genes that increase cancer risk twofold when the allele frequency is 5%, and more than 1500 would still be required when the allele frequency is 25%.[41] Changes in cancer incidence over the course of the 20th century; many reflecting behavior patterns (e.g., lung cancer in smokers), infectious diseases (e.g., stomach cancer in people infected with *H. pylori*), or exposure to special cultural factors such as urbanization or diet (e.g., increasing breast cancer rates in the second and subsequent generations of Asian-American women) underscore the significant influence that the environment exerts on the genetic makeup of any individual.[41-43] We anticipate that the combination of novel molecular and epidemiological approaches, combined with the judicious use of laboratory animal models and naturally occurring cancers of humans and companion animals, will allow us to tease apart the relative contributions of genes and environment to the process of carcinogenesis in the near future.

## Tumor Stem Cells: Implications for Therapy

The progressive mutations leading to cancer, along with selection and genetic instability, would appear to provide sufficient reasons for the incredible genetic heterogeneity found in most tumors. However, this would assume that all tumor cells possess an equal capacity for self-renewal and that proliferation is a stochastic ("random") process driven entirely by environmental selection of favorable mutations. Until recently, this was the dominant hypothesis for the origin and progression of tumors, and it may have been reinforced by the success of somatic cloning experiments that demonstrated nuclear "de-differentiation" was not only theoretically

possible but also could be used in practice for reproductive cloning.[44] However, a competing hypothesis to explain these observations is that self-renewal is limited to a small population of "cancer stem cells," similar in many ways to normal pluripotent stem cells that give rise to a population of lineage-committed cells that form organs and tissues.[45] The existence of such tumor stem cells is now documented; they are characterized both by peculiar phenotypes and by defined sets of mutations of WNT, MLL, HOX family members, and a small number of other genes.[45,46] These stem cells promote an environment that resembles wound healing,[46] except that in the case of the tumor, the proliferative and expansive process is not extinguished and the tissue never heals. The existence of cancer stem cells is not simply a matter of intellectual curiosity, as they can significantly impact therapeutic responses.[47] Dean and colleagues proposed four models that may account for resistance to therapy and subsequent relapse (Figure 1-4). In the "conventional" model, a cell acquires a drug resistant phenotype due to random mutations that promote expression of efflux-pump proteins (MDR), and this becomes the dominant phenotype by selective pressure exerted upon exposure to chemotherapy. In the second model, the tumor contains a small number of stem cells that express drug transporters a priori (as a component of their normal phenotype) and that undergo selection in a similar fashion to the cells that acquire drug resistance in the first scenario. In the third model, cancer stem cells acquire the drug-resistant phenotype through a similar process as that described in the first scenario, the main difference being that cancer stem cells retain high proliferative potential, whereas the proliferative capacity of lineage-committed progeny is limited. In the final model, both stem cells and progeny are inherently resistant to drugs and targeted therapy. Whereas the first three models predict variable levels of response and relapse, a clinical response would not be observed in the fourth model. Ultimately, elements from both the stochastic theory and the stem cell theory may prove to be correct.

From Concepts to Clinics: Molecular Cytogenetics Usher an Era of Opportunity

Improvements in our fundamental understating of cancer biology present opportunities to lower the morbidity and mortality associated with this disease. Since the 1960s, conventional cytogenetic approaches provided clues to the genetic basis of cancer; more recently, molecular cytogenetics gave us tools to identify nonrandom numerical and structural chromosome aberrations, which by extension offer insights into specific genes that contribute to the origin and progression of cancer. Numerical changes
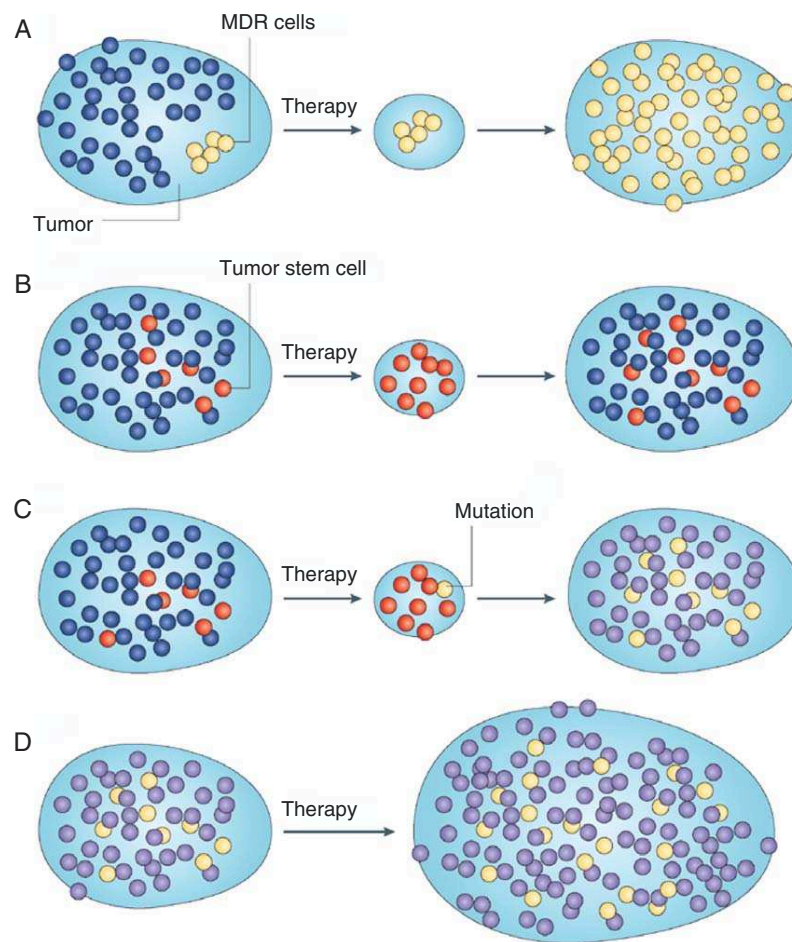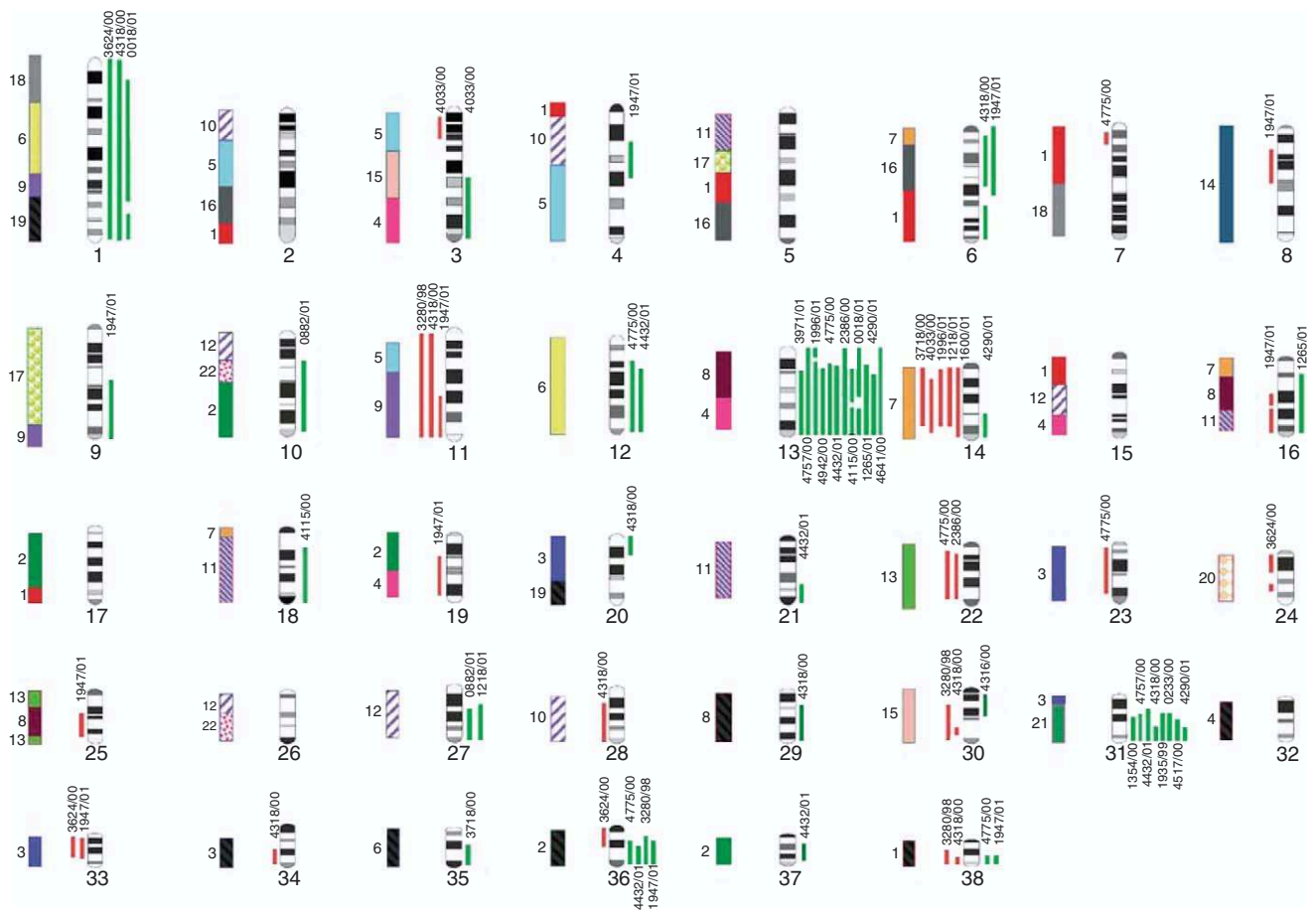
Figure 1-4

Models of tumor drug resistance. **(A)** In the conventional model of tumor-cell drug resistance, rare cells with genetic alterations that confer multidrug resistance (MDR) form a drug resistant clone (yellow). Following chemotherapy, these cells survive and proliferate, forming a recurrent tumor that is composed of offspring of the drug-resistant clone. **(B)** In the cancer-stem cell model, drug resistance can be mediated by stem cells. In this model, tumors contain a small population of tumor stem cells (red) and their differentiated offspring, which are committed to a particular lineage (blue). Following chemotherapy, the committed cells are killed, but the stem cells, which express drug transporters, survive. These cells repopulate the tumor, resulting in a heterogeneous tumor composed of stem cells and committed but variably differentiated offspring. **(C)** In the "acquired resistance" stem-cell model, the tumor stem cells (red), which express drug transporters, survive the therapy, whereas the committed but variably differentiated cells are killed. Mutation(s) in the surviving tumor stem cells (yellow) and their descendants (purple) can arise (by mechanisms such as point mutations, gene activation or gene amplification), conferring a drug-resistant phenotype. As in model **A**, the stem cell with the acquired mutations could be present in the population before therapy. **(D)** In the "intrinsic resistance" model, both the stem cells (yellow) and the variably differentiated cells (purple) are inherently drug resistant, so therapies have little or no effect, resulting in tumor growth. (*Reproduced with permission from M. Dean et al., "Tumor stem cells and drug resistance,"* Nature Reviews—Cancer 5:275–284, 2005. *©Nature Publishing Group.*)

represent a deviation from the normal gene copy number, leading to increased or decreased expression of genes, most often genes that regulate growth or survival (positively or negatively). Structural changes result in genome reorganization, which may cause genes that are separated in the *normal* genome to be brought into close proximity in the *tumor* genome with consequent effects on gene dysregulation.

Clonal chromosome aberrations have been identified in almost 27,000 human neoplasms representing 75 different types of cancer (see http://cgap.nci.nih.gov/Chromosomes/Mitelman). Among domestic animals,

Figure 1-5

Composite of CGH profiles from 25 canine lymphoma cases. The canine DAPI-banded ideogram is displayed. For each case, genomic gains and losses are shown as green and red bars to the right and left of each chromosome, respectively. Each vertical bar represents a site of genomic imbalance in a single case (labels at the top or bottom of red/green bars represent individual cases) and demonstrates the physical extent of the chromosome over which the aberration was detected. The evolutionarily conserved chromosome segments shared with the human karyotype are identified with colored bars to the far left of each chromosome. *(Reproduced with permission from R. Thomas et al., "Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridization and a panel of single locus probes,"* British Journal of Cancer *89:1530–1537, 2003. ©Nature Publishing Group.)*

progress has allowed us to develop a canine molecular cytogenetics "toolbox," which will accelerate progress in our understanding of the genetics underlying dog tumors. For example, comparative genomic hybridization (CGH) analysis has allowed investigation of numerical chromosome aberrations in canine tumors (Figure 1-5).[48–51] Similarly, fluorescence *in situ* hybridization (FISH) analysis has allowed the precise definition of structural chromosome aberrations that show a convincing evolutionary history between homologous cancers of dogs and people.[52] These analyses can be used to predict both

the genetic origin and the response to therapy of common tumors, such as canine lymphoma. For instance, gain of canine chromosome 13 (CFA 13), particularly in a region syntenic to human chromosome 4q (HSA 4q), occurs in ~70% of canine diffuse B cell lymphoma.[51] This suggests that this region of the genome contains heretofore-unidentified genes that are etiologically and prognostically significant for this disease. Gain of CFA 13 also is predictive for chemotherapy response in dogs,[51,53] perhaps because amplification of the c-*Myc* and c-*Kit* oncogenes, both of which are encoded in this region of

CFA 13, increases the proliferative rate of the malignant cells and consequently their susceptibility to antimitotic compounds. This abnormality appears to supersede other changes that are indicators of poor prognosis, such as loss of CFA 11, although this remains to be confirmed in larger controlled clinical studies.

SUMMARY

We can confidently state that the genetic basis of cancer is now beyond question. It is estimated that at least five mutational events are required for overt malignant transformation, and genomic instability seems to be necessary to establish a self-renewing population of cells (possibly cancer stem cells) whose progeny expand to cause clinical disease. Ultimately, a subpopulation that is endowed with metastatic properties and is drug resistant leads to death of the cancer patient. A major focus of contemporary cancer genetics is to define whether such properties are inherent to cancer stem cells or whether they arise by natural selection and clonal evolution. Current knowledge and available molecular tools allow us to predict prognosis and response to therapy in some cancers of companion animals, and we believe the availability and usefulness of such tools in clinical practice will expand rapidly. Hence, as we improve our understanding of fundamental mechanisms that account for malignant transformation and tumor progression, we will be able to design even better strategies for cancer prevention and therapy.

ACKNOWLEDGMENTS

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SECTION B
Chemical, Physical, and Hormonal Factors

Carolyn J. Henry

In 1978, the United States Congress ordered the development of the first Report on Carcinogens (RoC), a document designed to educate the public and health professionals on potential cancer hazards. The document is now required by law to be released every 2 years by the Secretary of the Department of Health and Human Services. The most recent RoC, released in January 2005, lists 246 potential carcinogens, of which 58 are categorized as *known to be human carcinogens* and 188 are categorized as *reasonably anticipated to be human carcinogens*.[1] While no such report exists for companion animals, it is reasonable to assume that considerable overlap would exist between such a list and the potential carcinogens found in the RoC. This most recent RoC, the Eleventh Edition, is the first to include neutrons, x- and gamma-radiation and viruses (human papillomavirus, Hepatitis B virus, and Hepatitis C virus). Although the list of carcinogens associated with cancer in companion animals is somewhat shorter, this section will address chemical, physical, and hormonal factors thought to be associated with carcinogenesis in pet animals. Viral carcinogenesis will be addressed in a separate section (see Viral Factors in Section A).

CHEMICAL FACTORS

Environmental Tobacco Smoke

Despite ample evidence that secondhand smoke increases the risk of lung cancer in people, the data for this effect in companion animals is less compelling.[2,3] One case-control study involving canine lung cancer cases from two veterinary hospitals showed a weak relationship between living with a smoker and development of lung cancer, and the risk did not increase with an increased smoke exposure index.[4] However, evidence for a relationship between exposure to environmental tobacco smoke (ETS) and development of other malignancies in companion animals is mounting.

Based on evidence from some human reports that smoking may increase the risk of non-Hodgkin's lymphoma,[5,6] Bertone et al. examined the relationship between ETS exposure and development of feline lymphoma.[7] They conducted a case-control study of 80 cats with malignant lymphoma and 114 control cats with renal disease that presented to Tufts University School of Veterinary Medicine between 1993 and 2000. They reported that the relative risk of lymphoma for cats with any household ETS exposure was 2.4. As has been reported for male smokers,[8] the risk of lymphoma amplified with both increased duration and quantity of exposure.

Hypothesizing that inhalation and ingestion of carcinogenic compounds in ETS during grooming might

also predispose cats in smoking households to development of oral squamous cell carcinoma (SCC), the same group examined ETS, as well as other environmental and lifestyle risk factors, in cats with SCC.[9] The study population consisted of 36 cats with histologically confirmed oral SCC, and their control population consisted of 112 cats with renal disease, all presenting to Tufts University between 1994 and 2000. Exposure to ETS was associated with a twofold, but not statistically significant, increased risk of oral SCC.[9] Interestingly, in a separate report, the investigators showed that SCC tissue from cats exposed to any ETS was 4.5 times more likely to overexpress p53, and cats with 5 years or more of ETS exposure had tumors that were 7 times more likely to overexpress p53.[10] Although the findings did not reach statistical significance, the collective work of this group provides an intriguing suggestion that both ETS and p53 gene mutations may play a role in the etiology of feline oral SCC.

Pesticides, Herbicides, and Insecticides

In 1991, investigators at the National Cancer Institute (NCI) completed a case-control study to examine the relationship between exposure of dogs to the herbicide 2,4,dichlorophenoxyacetic acid (2,4-D) and development of lymphoma.[11] Dogs with a confirmed histological diagnosis of lymphoma during a 4-year period were identified through the computerized medical record information from three veterinary teaching hospitals. Each case animal was age-matched with two control animals. The first control group consisted of dogs diagnosed with tumors other than lymphoma during the same time period, and the second control group was a nontumor control group, selected from all other dogs presenting to the hospital for conditions deemed unrelated to chemical exposure. Owners were questioned about household use and potential exposure to chemicals, including commercial lawn care and owner-applied herbicides. The investigators reported a positive association between exposure to owner-applied 2,4-D or the use of commercial lawn care services and the development of canine lymphoma. The risk of lymphoma development doubled when owners applied 2,4-D liquid or granules to the lawn four or more times a year.

Following release of these findings, an independent review panel convened to assess the validity of the study.[12] This panel voiced concerns about the study design, data analysis, and interpretation, concluding that a relationship between 2,4-D exposure and the development of canine lymphoma could not be established based on the study by Hayes et al. The original investigators subsequently reanalyzed their data, addressing many of the concerns raised by the scientific review panel.[13] In their second study, Hayes et al. used a more stringent definition of exposure to 2,4-D, including only cases where the owner applied 2,4-D as the sole herbicide and did not use other lawn chemicals or lawn care services. Their second report in 1995 did not show a statistically significant association between exposure to 2,4-D and development of lymphoma. However, they concluded that their results did indicate a dose-response relationship between disease incidence and number of yearly 2,4-D applications by dog owners. A fourth report was published in 1999 in which researchers at Michigan State University obtained the original 1991 data and reanalyzed it, using the more stringent definition of exposure and completed a dose-response analysis. This study, which was funded by a grant from a chemical industry task force, showed no dose-response relationship between number of 2,4-D applications and the occurrence of canine lymphoma.[14] Although increased urinary excretion of 2,4-D has been demonstrated in dogs exposed to herbicide-treated lawns, a direct link between such exposure and development of lymphoma has not been shown.[15] A more recent case-control study conducted in Italy was designed to assess the effect of residential exposure to environmental pollutants on the risk of developing lymphoma in dogs.[16] The investigators were unable to demonstrate an association between exposure to pesticides (which by their definition included herbicides) and development of canine lymphoma. They did, however, find that living in industrial areas and owner use of chemicals such as paints and solvents were significantly and independently associated with lymphoma.

Transitional cell carcinoma (TCC) of the bladder is another malignancy that has been linked to insecticide and herbicide exposure. In a 1989 case-control study by Glickman et al., 59 dogs with TCC and 71 age- and breed size-matched control dogs with other neoplasms or chronic disease were compared to assess the effect of obesity, exposure to sidestream (secondhand) cigarette smoke and chemicals, and use of topical insecticides on risk of TCC.[17] They reported an increased risk of TCC in dogs treated with topical insecticides, with an enhancement of this risk in overweight or obese dogs. In the aforementioned study of risk factors for oral SCC in cats, Bertone et al. reported a significantly increased risk of oral SCC in cats that wore flea collars.[9] However, newer topical spot-on flea and tick products have been evaluated in populations of Scottish terriers, a breed known to be at risk for development of TCC of the urinary bladder, and have not been shown to increase the risk of TCC.[18] Similar studies in Scottish terriers have suggested that exposure to lawn and garden care products containing phenoxy herbicides, including 2,4-D, 4-Chloro-2-methylphenoxy acetic acid (MCPA), and 2-(4-chloro-2-methyl) phenoxy propionic acid (MCPP), is associated with an increased risk of TCC.[19] While it is difficult to prove a link between phenoxy herbicides and development of lymphoma or TCC, attempts to minimize the access of pets to these products would be prudent.

Cyclophosphamide

The cytotoxic alkylating agent, cyclophosphamide, has been implicated in the development of urinary bladder cancer in both humans and dogs.[20–22] A potential side effect of cyclophosphamide therapy is sterile hemorrhagic cystitis, which may develop due to the irritating effects of the drug's metabolite, acrolein, on the bladder mucosa. Although the specific etiology is unknown, it is speculated that chronic inflammation secondary to acrolein exposure is the underlying event that leads to bladder cancer in some patients.

Rural versus Urban Environment

Although several reports have evaluated differences in companion animal cancer incidence between rural and urban settings, the underlying cause for these differences is unclear. An increased incidence of some canine cancers, including lymphoma, tonsillar SCC, and nasal carcinoma,[16,23,24] have been reported in urban/industrial settings as compared to rural settings. However, the coexistence of multiple environmental carcinogens in the same setting makes discerning the "smoking gun" a difficult task. Nonetheless, the study of animals as sentinels of environmental health hazards has been recommended and provides supportive evidence for carcinogenic risk assessment across species.[25–27]

PHYSICAL FACTORS

Sunlight

One of the least controversial relationships between potential causative agents and development of cancer is that of sunlight or ultraviolet irradiation and subsequent development of skin cancer. Recognized for its role in human squamous cell carcinoma induction, sunlight has also been implicated as a cause of SCC in domestic animals and livestock, an implication that is strengthened by a clear dose-response relationship shown in both epidemiological and experimental studies.[28–31] In particular, light skin pigmentation and chronic sun exposure are associated with development of facial, aural, and nasal planum SCC in white or partially white cats (Figure 1-6) and may also play a similar role in some cutaneous SCC lesions in dogs and cutaneous hemangiosarcomas in laboratory beagles. The portion of the ultraviolet spectrum most likely to be responsible for nonmelanotic skin lesions in people and animals is ultraviolet B (UV-B), which is in the range of 280 to 320 nm.[28] Cumulative long-term exposure to UV-B may induce skin tumors directly through genetic mutations, including mutations in p53, and indirectly by impairing the response of the immune

Figure 1-6

Squamous cell carcinoma of the nasal planum. This cancer is linked with light skin pigmentation and sun exposure in cats. *(Photo courtesy of Kim Selting.)*

system to tumor antigens.[28,32,33] Pets are at greatest risk of exposure to UV-B during the midday hours and should be protected from this exposure, especially if they are of a lightly pigmented breed.

Trauma/Chronic Inflammation

Chronic inflammation may lead to mutations that result in neoplastic transformation. In four dogs with chronic pigmentary keratitis, neoplastic lesions of the cornea, including three SCC and one squamous papilloma, were reported.[34] Although the underlying etiology of the keratitis could not be confirmed, the neoplastic transformation was likely related to chronic inflammation. Earlier reports have linked feline eye tumors to ocular trauma and secondary uveitis and lens rupture.[35] Unlike the corneal tumors reported in dogs with pigmentary keratitis, the ocular lesions in cats were intraocular sarcomas. Despite the varied histology, in all cases, the underlying etiology was thought to be related to inflammatory changes. Another small animal malignancy thought to be associated with inflammation is vaccine associated feline sarcoma (VAFS). This tumor type and its etiology are discussed in detail in Chapter 20.

Magnetic Fields

In the late 1970s, a potential link between childhood cancer and chronic low-dose exposure to magnetic fields was proposed.[36] Since that time, multiple studies have attempted to discern links between magnetic fields and a variety of human cancers from hematopoietic malignancies to breast cancer. The extremely low frequency (<60 Hz) magnetic fields in question are ubiquitous in today's society and are generated by household appliances, industrial machinery, and electrical power

lines. In that pets share our environment and have similar exposure to magnetic fields, a similar risk of cancer development has been proposed for companion animals. In a 1995 study, the risk for canine lymphoma was found to be highest in dogs from households with the highest measured exposure to magnetic fields.[37] The risk was related to both duration and intensity of exposure. Risk was increased for dogs that spent more than 25% of the day outdoors. In the following year, a report was published by the National Research Council (NRC) at the request of Congress, which reviewed more than 500 studies on the subject of cancer risk and exposure to electromagnetic fields.[38] The report concluded that, although a weak association has been shown between development of childhood leukemia and exposure to electromagnetic fields, no clear evidence exists to suggest that exposure to electromagnetic fields is a true threat to human health. To the author's knowledge, no further reports on the possible link between magnetic fields and cancer in companion animals have been published, although the magnetic field debate continues in the human literature. The NRC report suggested that other factors including air quality and proximity to high traffic density may be more likely environmental causes of cancer than low-frequency magnetic fields.

Radiation

The first report of cancer development after therapeutic irradiation in dogs dates back to the early 1980s, when orthovoltage radiation was considered state of the art.[39] At that time, the term "malignant transformation" was used to describe the development of epithelial malignancies at the site of prior irradiation for acanthomatous epulides in four dogs. With the benefit of hindsight and review of more recent cases, the author of the original report has suggested that the concept of "malignant transformation" should be discarded, in that the occurrence of second tumors was not likely due to a true transformation of epulides into carcinomas.[40] Rather, radiation carcinogenesis is likely the cause of a second, unrelated tumor in radiation fields. In human medicine, most tumors occurring in heavily irradiated treatment fields are of mesenchymal, rather than epithelial, origin.[41,42] Several reports of sarcomas occurring in sites of prior radiation can be found in the veterinary literature.[39,43–45] In a retrospective review, McEntee et al.[40] described the development of a second tumor (one sarcoma and one osteosarcoma) in two of 57 dogs undergoing definitive megavoltage radiation therapy with [60]Cobalt photons for acanthomatous epulis. These tumors occurred 5.2 and 8.7 years after the initial treatment. The overall incidence of second tumors was lower in this study than in previous reports (3.5% versus up to 18%).[39,46] The fact that no epithelial tumors were reported may indicate a more efficient targeting of a subpopulation of malignant epithelial cells by megavoltage radiotherapy, as compared to orthovoltage. Large doses per fraction are more closely linked with sarcoma development. The report suggests that the risk of second tumors at sites of radiation therapy is primarily of clinical concern for young dogs expected to enjoy long-term survival following treatment of their primary tumor. Second tumors have also been reported in at least six people who have undergone stereotactic, high-dose single fraction radiosurgery.[47] As this radiation technique becomes more commonplace in veterinary medicine, the possibility of second tumors may need to be considered in companion animals as well.

Surgery and Implanted Devices

The development of sarcomas at the site of metallic implants has been reported in people, dogs, and laboratory animal models.[48,49] However, it is often difficult to discern if sarcoma development is related to fracture fixation devices or to other factors including wound healing complications and osteomyelitis. The largest veterinary study examining the relationship between metallic implants and tumor development in dogs was published in 1993 by Li et al.[49] The authors reported on 222 dogs that developed tumors of any kind after fracture fixation, compared to 1635 dogs that underwent fracture fixation without subsequent tumor development. They concluded that use of metallic implants was not a risk factor for bone tumor development. Other types of implants and foreign materials related to surgery are sporadically implicated in carcinogenesis in human and veterinary case reports. Published examples include one dog that developed a myxoma at the site of a subcutaneous pacemaker implantation and another that developed a jejunal osteosarcoma associated with a surgical sponge presumably not retrieved during an abdominal surgery 6 years prior.[50,51]

Asbestos

Asbestos exposure has been established as a risk factor for development of mesothelioma in people,[52] with between 60% and 88% of all cases attributable to asbestos exposure.[52] A similar association has been found for dogs whose owners have an asbestos-related occupation or hobby.[53] This association is further supported by a study in which significantly more asbestos bodies were found in dogs with mesothelioma than in control dogs.[54] Pericardial mesothelioma has been reported in five golden retrievers with long-term histories of idiopathic hemorrhagic pericardial effusion, suggesting that other factors, including breed predispositions and nonasbestos-related causes of chronic inflammation, should also be considered in the etiology of mesothelioma affecting the pericardium.[55]

HORMONAL FACTORS

Estrogen and Progesterone

Canine Mammary Cancer

When considering hormonal factors associated with cancer in domestic animals, perhaps the best-established model is that of canine mammary cancer. Mammary tumors are the most common neoplasm of female intact dogs, affecting approximately 260/100,000 dogs in the United States each year.[56,57] It is well established that dogs spayed before their first estrous cycle have a greatly reduced risk of developing breast cancer.[58] The risk of developing mammary tumors, approximately 50% of which are malignant, rises from nearly 0% in dogs spayed prior to their first estrus to 26% for dogs that are spayed after their second estrus.[58,59] Mammary tumors primarily affect middle-aged (9 to 11 years) female intact dogs, with an increased incidence beginning at approximately 6 years of age.[60] Sexual steroid hormones are thought to have their primary effect on target cells during the early stages of mammary carcinogenesis in dogs, as supported by the lack of protective effect with spaying after two estrous cycles and the presence of estrogen and progesterone receptors in higher proportions in normal breast tissue and benign lesions, as compared to malignant breast cancer tissue or metastatic lesions.[60–67] In addition to the influence of ovarian hormones on breast cancer development, the use of products containing medroxyprogesterone acetate (progestin and estrogen combination) to prevent estrus or to treat pseudopregnancy in dogs has been linked to an increased incidence of mammary tumor development.[68–70]

Since the early 1990s, it was noted that progestin-induced growth hormone (GH) excess in dogs originates in the mammary gland. Within the mammary gland, the gene encoding GH may act in an autocrine/paracrine fashion, effecting cyclic epithelial changes and, perhaps, carcinogenesis. Investigations to determine the mechanism of progestin-induced mammary GH expression in dogs has led to the cloning and cellular localization of the canine progesterone receptor (PR).[71] The investigators concluded that within the same mammary gland cell, the activated PR may transactivate GH expression and function as a prerequisite transcription factor. However, this regulation may be lost during malignant transformation. Mammary GH expression has been reported in people as well, suggesting that evaluation of links between this hormone and mammary carcinogenesis may have implications for both species.[72,73]

Feline Mammary Cancer

In feline mammary cancer development, both estrogen and especially progesterone are thought to play important roles, although the underlying mechanisms are less clear. Prior studies have indicated that intact female cats and those cats exposed regularly to progestin are at an increased risk for mammary cancer development. The literature also suggests that, as is the case in dogs, ovariectomy may be protective against mammary gland tumor development in cats.[56,74,75] In one study, cats ovariectomized at 6 months of age had an approximate sevenfold reduction in risk of mammary tumor development compared to intact cats.[56] What has been lacking in the veterinary literature is an epidemiological study of cats with age-matched controls for comparison, to specifically investigate the effects that spaying and age of spay have on the risk of feline mammary carcinoma development. What has been lacking in the veterinary literature is an epidemiological study of cats with age-matched controls for comparison, to specifically investigate the effects that spaying and age of spay have on the risk of feline mammary carcinoma development. Overley, et al., recently attempted to address these issues by reporting results of a retrospective study that compared a population of 308 cats with biopsy-proven mammary carcinoma diagnosed between 2000 and 2001 and a control population of 400 female cats not diagnosed with mammary tumors, but from the same biopsy service population as the affected cats. Cats from the two groups were frequency-matched by age and year of diagnosis.[76] The study reported a statistically significant reduction in mammary cancer risk for cats spayed prior to one year of age, with a 91% reduction in risk for those spayed prior to 6 months of age and an 86% reduction in risk for those spayed prior to one year of age, compared to intact cats. Although the study was retrospective in nature and relied on questionnaire data from a survey with a 58% response rate, the manuscript is the first published report attempting to age-match controls and evaluate age at time of spay as a risk factor for mammary tumor development in cats. Although further epidemiological evaluation and prospective assessment are needed to confirm these findings, the reported results provide justification for recommending ovariohysterectomy prior to 1 year of age in cats.

Lymphoma

Surveillance, Epidemiology and End Results (SEER) data indicate that non-Hodgkin's lymphoma (NHL) is approximately 50% more common among men than women.[77] Although a similar male predisposition is reported for canine lymphoma, the underlying role of gender in lymphoma etiology remains elusive. The author and others undertook a population-based study using the Veterinary Medical Database (VMDB) and the Veterinary Cancer Registry (VCR) (www.vetcancerregistry.com) to determine the relationship between gender and development of canine lymphoma.[78] Data from 1980 to 2000 were retrieved from the VMDB and sorted by gender and reproductive status. In the statistical analysis, spayed or neutered dogs diagnosed with lymphoma were compared to intact dogs seen each year in each gender category. The VCR was searched for all canine

lymphoma cases, and the number of cases in each gender group was compared to the total number of cancer diagnoses per group. The VMDB included nearly 15,000 lymphoma cases in a population of 1.2 million dogs, and in the VCR database, 394 lymphoma cases were identified among 6070 canine cancer diagnoses. In both analyses, intact females were significantly less likely to develop lymphoma than were other gender groups. Based on this initial data, it is proposed that further examination of the role of estrogen in the development or prevention of canine lymphoma is warranted.

Androgens/Testosterone

Perianal Adenoma

Perianal adenomas are considered to be androgen dependent, occurring primarily in male intact dogs, as opposed to perianal adenocarcinomas, which occur in both intact and castrated males. Perianal adenomas may also occur in female dogs secondary to testosterone secretion from the adrenal gland.[79] The partial or complete resolution of the majority of these tumors in male dogs following castration further supports the assertion that androgens are involved in the etiology of this tumor.[80]

Prostate Cancer

In contrast to the well-established link between testosterone and development of benign prostatic hyperplasia in dogs and man, prostatic adenocarcinoma does not occur in increased proportions in intact dogs, as compared to castrated dogs.[81] In fact, some studies have demonstrated an increased risk of prostate cancer in neutered dogs, with the supposition that castration is not an initiating event but can favor tumor progression.[82–85] A clear relationship between age at castration and risk of hormone dependent prostatic adenocarcinoma development is yet to be determined.

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SECTION C
Cancer-Causing Viruses

Dennis W. Macy

Both deoxyribonucleic acid (DNA)- and ribonucleic acid (RNA)-containing viruses are known to cause cancer. An initial step in malignant transformation of normal cells by most tumor viruses is integration of all or part of the viral DNA (or DNA copy of retroviral RNA) into the host cell genome. For some viruses, specific viral genes (oncogenes) have been identified that lead to malignant transformation when expressed in normal cells. Other viruses, through the process of integration, activate the expression of normal cellular genes, leading to overexpression or inactivation of genes, resulting in cellular transformation or uncontrolled growth.[1]

TUMOR-CAUSING VIRUSES OF DOGS

Papillomaviruses

Papillomaviruses are oncogenic, contagious, and infectious and have been described in several animal species.[2] Papillomaviruses are considered species specific, and isolates of humans, cattle, and dogs lack serologic cross-reactivity.[2] However, cross-infection of similar species can occur. For example, the coyote can be infected with dog isolates, and bovine papillomaviruses type 1 and type 2 have been reported to infect horses.[3]

Four or possibly more papillomaviruses infect dogs and are responsible for a wide spectrum of clinical syndromes. Papillomaviruses of the family Papovaviridae produce benign, mucocutaneous, and cutaneous canine papillomas and in rare cases transform into squamous cell carcinomas.[4,5]

The canine papillomaviruses are naked DNA viruses; they are larger than the canine parvoviruses but similar in structure. Electron microscopy has been used to detect the virus in infected tissues. Like other papillomaviruses, canine papillomaviruses are resistant, acid stable, and relatively thermostable.[6] Only a limited sequence homology exists between the DNA sequences of papillomaviruses of different species, but substantial sequence homology exists between isolates from any given species.[2]

Pathogenesis

Papillomas develop subsequent to introduction of papillomavirus through breaks in the epithelium. Different viruses derived from the same species are believed to correlate with the type of clinical disease produced by the virus (i.e., oral versus cutaneous isolates). However, this feature of papillomaviruses has yet to be proven for the dog, and experimentally, ocular isolates have produced oral papillomas.[7-11] The presence and location of mature, complete viruses on the surface of papillomas are believed to aid its transmission to adjacent epithelial tissues.[2] In contrast to other oncogenic or transforming DNA viruses, papillomaviruses rarely integrate into the cellular genome and remain episomal.[2]

Infection of epithelial cells results in a marked increase in cellular mitosis and hyperplasia of cells with a strand of spongiosum, with subsequent degeneration and hyperkeratinization.[12] Clinical evidence of hyperplasia

and hyperkeratinization usually manifests 4 to 6 weeks after infection.[12] Canine papillomas generally persist for 4 to 6 months in the mouth and 6 months to 1 year on the skin before undergoing spontaneous regression, and multiple warts generally regress simultaneously.[12] Although antibodies are produced against the papillomavirus, antibody levels do not appear to correlate with either growth or regression of the papilloma; the mechanism of induction or regression remains unknown, although it is thought in most cases to be associated with cellular immunity.[13]

Clinical Features

Papillomas may be referred to as *warts, verruca vulgaris, squamous cell papillomas*, or *cutaneous papillomatosis*. Papillomas caused by the papillomavirus usually are multiple and frequently infect young dogs. In the dog, multiple papillomatosis most frequently is seen in the oral cavity, involving the labial margins, tongue, pharyngeal mucosa, hard palate, and epiglottis (Figure 1-7).[13] Four to 8 weeks after infection, small, pale, smooth, elevated lesions appear; these quickly develop a cauliflower-like appearance, with fine, white frons extending from the surface of the lesions. Multiple sites of susceptible tissue in the oral cavity appear to be affected early in the course of the disease; as many as 50 to 100 tumors may be present at the time of diagnosis.[13] The primary complaints of owners of infected dogs are halitosis, ptyalism, hemorrhage, and difficulty eating. Most oral cavity papillomas start regressing after 4 to 8 weeks. However, some oral lesions may show incomplete regression, and some have been known to persist up to 24 months.[13]

Ocular papillomas, which are less numerous than the oral type, appear on the conjunctiva, cornea, and eyelid margins (Figure 1-8). Ocular papillomas also occur less often than oral lesions.[13] Experimentally, viruses isolated from ocular lesions can produce oral papillomatosis, although whether this occurs in nature is unknown.[7] Ocular papillomatosis most frequently occurs in dogs 6 months to 4 years of age, but it occasionally is reported in older dogs.

Multiple cutaneous papillomatosis is thought to be of viral origin (Figure 1-9); however, evidence suggests that it is not the same strain of papillomavirus that produces oral papillomatosis in the dog.[7] Multiple skin papillomatosis affects a much broader age range of

Figure 1-8

Solitary ocular papilloma in a dog.

Figure 1-7

Multiple papillomatosis in the oral cavity of the dog.
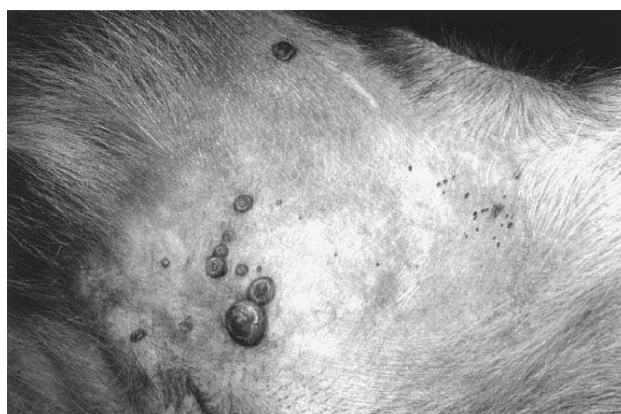
Figure 1-9

Multiple cutaneous papillomatosis in the inguinal region of a dog.

canine patients, and regression of the lesion is prolonged, sometimes taking years.[13] A rare form of cutaneous papillomatosis, in which the lesion appears in the interdigital areas of the pad, has been described in greyhounds, particularly young ones (12 to 18 months of age).[11] Canine pigmented plaques have been associated with papillomaviruses in miniature schnauzers and pugs.[14] The lesions may or may not regress and are considered premalignant.

Although papillomatosis should be considered a benign disease, in rare cases oral and corneal papillomas have transformed into squamous cell carcinomas.[4,5]

Treatment

Most clinicians elect not to treat papillomatosis because of the lack of proven efficacy of recommended treatments and the expected spontaneous regression of these tumors. However, if the number of papillomas increases or if the animal has significant difficulty eating, owners often request treatment. Surgical excision, cryosurgery, laser surgery, or electrosurgery for just a few lesions has resulted in regression of the remaining papillomas, presumably through immunologic mechanisms.[13,15,16] The exact mechanism by which regression of papillomas occurs is unknown. Serum from dogs in which papillomas have undergone spontaneous regression not only fails to produce tumor regression when administered to infected animals, it actually enhances existing tumor growth. However, administration of immune lymphocytes from dogs in which tumors have regressed has been shown to enhance regression.[6] This effect may be a result of induction of blocking antigen-antibody factors, which may impede cytotoxic lymphocyte action on target cells. CD4 lymphocytes activate macrophages and have been shown to inhibit the virus in dogs.[6] Interferon also has been tried, with some success (1 to 3 million IU/m² given subcutaneously three times a week [Monday-Wednesday-Friday]), and chemotherapy of resistant lesions has produced variable results.[17] Corticosteroids should be avoided because they are thought to contribute to the dissemination of papillomas.[18]

Most systemic chemotherapeutic agents (e.g., bleomycin, vincristine, cyclophosphamide, and doxorubicin) have failed to cause regression of papillomas. However, etretinate (1 mg/kg given orally daily) has been effective in some dogs with persistent papillomas.[19,20] Topical or intralesional compounds containing 5-fluorouracil (5-FU) have been used in both humans and dogs to treat papillomas. In the past, autogenous wart vaccines have been recommended but have proved of little value in the treatment of resistant papillomatosis of the dog.[13] In at least one study, papillomavirus vaccines have been shown to prevent the development of oral papillomas in the dog; however, cutaneous neoplasms at the injection sites have been attributed to administration of the vaccines.[10,21,22]

TUMOR-CAUSING VIRUSES OF THE CAT

Papillomaviruses

Feline viral papillomatosis is a rarely recognized condition caused by a papillomavirus specific to the cat. The feline isolate is genomically very similar to canine isolates but is considered species specific. Papillomavirus-associated lesions have been reported in six species of felids besides the domestic cat: the mountain lion, Florida panther, bobcat, Asian lion, snow leopard, and clouded leopard.[23,24] Unlike in the domestic cat, in which the lesions commonly affect areas of haired skin, papillomas in exotic species most often are found in the oral cavity, similar to those in the dog.[23, 24] Despite the clinical similarities, genetic and antigenic studies indicate that each species of felid is infected by a unique papillomavirus.

Pathogenesis

In cats, as in other species, papillomas are believed to develop after the virus is introduced through lesions or abrasions in the skin. Unlike in the dog, most feline case reports involve older cats (6 to 13 years of age), although papillomavirus lesions have been reported in kittens 6 to 7 months old.[25,26] As in other species, impaired T-cell function likely plays a significant role in lesion formation. Papillomas in cats that are receiving immunosuppressive therapy or are infected with the feline immunodeficiency virus support this hypothesis.

Although papillomas most frequently are benign lesions, recent studies have associated the papillomavirus with squamous cell carcinomas and other malignant neoplasms in cats[27]; specifically, papillomavirus has been isolated from 30 of 63 squamous cell carcinoma *in situ* skin lesions. Through the use of polymerase chain reaction (PCR) techniques, papillomaviruses have been found in 17 of 19 and nine of 12 fibropapillomas in cats.[28,29] Although a cause-and-effect relationship has yet to be proven for carcinoma *in situ*, Bowen's disease, fibropapillomas, and papillomaviruses, the evidence is compelling.[27-29]

Bovine papillomaviruses may also play a role in the pathogenesis of feline cutaneous fibropapillomas. In a study of 20 cats with fibropapillomas, more than half were known to have exposure to cattle, and all were within an area with dairy farms.[28] In one isolate, the nucleotide sequence was similar to that of the bovine papillomavirus. Also, although papillomaviruses generally are species specific, an association between bovine papillomavirus types 1 and 2 has been suggested as causes of equine sarcoids.[30]

Clinical Features

Lesions in the cat differ from those in the dog, because they are more like plaques than warts. The plaques are

several millimeters in diameter, may be white or pigmented, and are scaly or greasy. Also, lesions in the cat usually affect haired skin rather than the mucous membrane locations common for oral and ocular papillomas of the dog and wild felids.[23,24]

Diagnosis

Definitive diagnosis depends on histopathologic, immunohistochemical, or electron microscope (EM) examination of excised lesions. Histologic features include proliferation of all cell layers with little or no inflammation. Typically the epidermal hyperplasia is accompanied by acanthosis, hypergranulosis, hyperkeratosis, and ballooning degeneration of cells of the stratum spinosum and stratum granulosum. Amphophilic cytoplasmic inclusion structures may be present in cells of the upper stratum granulosum. EM findings in the lesions include intranuclear particles within keratinized cells in the superficial epithelial strata of the plaques. Immunohistology can be performed on sections using band-reactive, genus-specific antiserum. Interestingly, the histologic features of the feline fibropapilloma are very similar to those of equine sarcoids, with characteristic fibroblastic proliferation, hyperplasia of epidermis, and rete ridges.[28] PCR also has demonstrated papillomavirus DNA in the lesions.

Treatment

Surgical excision is generally used, however, parenteral alpha interferon has been suggested as an alternative. *Medications containing 5-FU that are used in humans and dogs should not be used in cats.*

Retroviruses

Retroviral infections are considered the number one infectious cause of morbidity and mortality in the domestic cat. Before the vaccine was developed and routine testing and control measures became widespread, the feline leukemia virus (FeLV) was associated with one third of deaths in cats.[31,32] The cat is believed to be affected by the largest number of retroviruses of any companion animal, and these viruses produce a wide spectrum of diseases, including cancer.[33-35]

The cat has both endogenous and exogenous retroviruses. The endogenous retroviruses generally are considered nonpathogenic, are present in the host DNA, and are passed from generation to generation genetically, as are other chromosomal genes. The exogenous retroviruses include both pathogenic and nonpathogenic viruses and are passed horizontally and vertically between cats. Pathogenic exogenous retroviruses include FeLV and the feline immunodeficiency virus (FIV).[36] The exogenous RNA sequences of FeLV play the most important role in tumorigenesis in the cat.[35] Another pathogenic retrovirus, the feline sarcoma virus (FeSV), arises from the

combination of exogenous FeLV and proto-oncogenes in the cat's genome.[37] Feline syncytium-forming virus (FeSFV), also called the *feline foamy virus,* is a nonpathogenic exogenous retrovirus.[31]

FeLV is believed to have been contracted from the ancestral rat approximately 10 million years ago.[38] The ancestral source of other retroviruses is unknown. The three pathogenic retroviruses of clinical importance are FeLV, FIV and, in rare cases, FeSV.

Feline Leukemia Virus

The retrovirus FeLV belongs to the subfamily oncornavirus, or tumor-producing RNA viruses. Like other retroviruses, it has a single strand of RNA and an enzyme, reverse transcriptase (RT), that synthesizes DNA from the virus RNA template. Nondomestic felids, including the cheetah and bobcat, can be infected by FeLV; however, it is not considered enzootic in wild felids except for European wild cats in France and Scotland.[39,40]

The basic FeLV proteins include the envelope proteins and the core proteins, several of which are important clinically. Two envelope proteins, the P15E and the GP70 glycoproteins, have particular clinical significance.[41-43] P15E is thought to be one of the mediators of immunosuppression in FeLV-infected cats.[44] The glycoprotein of the envelope GP70 may contain three subgroup antigens, A, B, and C.[45,46] An individual cat may have combinations of viruses with these subgroup viral antigens. Considerable antigenic variation exists within subgroups, which can affect the biologic properties of the individual isolates or strains of FeLV.[35,45] These subgroup antigens bind the virion to receptors on the surface of cells. The specific characters of these proteins also predict the pathogenicity, host range, infectivity, and other biologic properties of the virus.[35,45] The antibodies produced against envelope proteins can be neutralizing and thus can prevent infection. Envelope proteins are very important components of FeLV vaccines.

Core proteins (capsids) include P15C, P12, P10, and P27. P27 is quite soluble and can be found in large amounts in the cytoplasm of cells and bodily fluids, such as tears and serum.[41-43] P27 is the antigen that is detected in immunofluorescent (IFA) tests and enzyme-linked immunosorbent assays (ELISAs), which are commonly used in the diagnosis of FeLV infection.[46]

Transmission

FeLV is an enveloped virus and is considered very fragile. Desiccation rapidly reduces the amount of viable virus in saliva, and inactivation occurs in 1 to 2 hours. In exudates or blood, the virus may be viable for only 48 hours (at 37° C) or 1 to 2 weeks (at 22° C).[47] Like most retroviruses, FeLV is rapidly inactivated by heating

and most disinfectants.[47] Given these characteristics, environmental contamination (e.g., examination tables, cages, and waiting rooms) is unlikely to be a potential source of FeLV infection.[35] Although saliva may contain up to 100,000 virus particles per milliliter, prolonged, intimate contact with infected cats usually is required for transmission. The factors most frequently incriminated in the transmission of FeLV are licking, biting, grooming, and sharing of litter pans, food bowls, and water dishes. Intimate contact is enhanced in catteries and multiple-cat households, where infection rates may be very high.[44]

Although cats may be infected with FeLV subgroups A, B, or C or other recombinants, only subgroup A has been found in cell-free fluids and is thought to be associated with natural transmission of FeLV. Subgroups B and C and other recombinants are more cell associated and are not thought to be transmitted in nature.[48-51]

Before vaccines and routine testing became available, the overall prevalence of FeLV infection in the United States was estimated at 1% to 3% of the population.[33,34] The prevalence of FeLV infection was less than 1% in single-cat households and as high as 30% in multiple-cat households.[52] The incidence of FeLV-positive test results in sick cats in the United States was approximately 11.5%.[55] Several studies have reported a decline in the prevalence of FeLV by as much as 50% over the past 20 years.[32,53,54]

The FeLV subgroups are characterized by their cross-interference with homologous but not heterologous subgroups of FeLV and by their host range and other factors. All naturally infected FeLV cats have subgroup A, 50% of infected cats have a combination of subgroups A and B, and 1% of infected cats in nature have a mixture of subgroup C either as AC or ABC.[33,55,56]

The relevance of subgroups in strains is essential to an understanding of the biodiversity of the clinical disease caused by FeLV infection. Although subgroups A, B, and C maintain 85% genomic homology, cats infected with various combinations of these subgroups may manifest vastly different diseases.

Subgroup A has a variety of strains that range from nonpathogenic to very pathogenic.[57] Although most strains of subgroup A have limited pathogenicity, if they are present with other subgroups, their pathogenicity increases dramatically.

Subgroup B is created when subgroup A recombines with endogenous FeLV envelopes at sequences already in the feline genome.[58-60] Each recombination is unique, resulting in many strains of FeLV-B. The combination of subgroups A and B is more contagious and pathogenic than subgroup A alone.[55-57] Cats infected with subgroups A and B often develop thymic lymphoma and myeloproliferative disease.[58]

Subgroup C arises from the mutation of subgroup A.[61] Cats may be infected with a combination of C and other subgroups, although these combinations are uncommon and are found in only about 1% of naturally infected cats. FeLV-C is antigenically similar to the associated membrane antigen (feline oncornavirus-associated cell membrane antigen [FOCMA]), and cats carrying FeLV-C have developed severe erythroid hypoplasia and anemia and usually die within 1 to 2 months.[48]

Further complicating the biodiversity of subgroups and stains is the fact that subgroups A and B can recombine with proto-oncogenes, such as *MYC* or *TCR*, producing FeLV-MYC or FeLV-TCR.[31] Both these recombinants are considered more potent tumor producers than their nonrecombinant FeLV parent. Another subgroup, T, is highly cytolytic for T lymphocytes and causes severe immunosuppression.[49-51]

The Rickard strain of FeLV (FeLV-R), although similar to MYC-containing recombinant strains in its ability to produce mediastinal lymphoma rapidly, does not recombine with the *MYC* gene.[31,62] Instead, it obtains some of the biologic effects by integrating adjacent to the *C-MYC* gene, causing its overexpression.[31]

Feline Oncornavirus-Associated Cell Membrane Antigen

FOCMA is a protein found on the surface of FeLV and FeLV-induced neoplasms but not on nonneoplastic feline cells.[63,64] FOCMA is detected serologically when cells expressing it react to immunoglobulins produced in cats that have regressed FeSV-induced fibrosarcoma or FeLV infection. The presence of FOCMA antibody is determined by the ability of the serum to react with FL74 cells, a transformed infected feline lymphocyte line.[65] Antibodies to FOCMA protect against neoplastic and myeloproliferative disease. Some FeLV vaccines contain FOCMA and elicit an anti-FOCMA response.[66] The relative importance of this in preventing disease in vaccinates is unknown.

Neoplastic Diseases Caused by FeLV

We have much to learn about the genetic basis for the vast diversity of tumor types produced by FeLV and its recombinants. We now know that FeLV, through one or another of its recombinants, may cause virtually any hematopoietic neoplasm in the cat. The only hematopoietic neoplasms not yet associated with FeLV in nature are mast cell leukemia, eosinophilic leukemia, plasma cell tumors, and polycythemia vera.[31]

Although FeLV infection is considered the most significant infectious cause of morbidity and mortality in cats, only 20% of cats persistently infected with FeLV develop lymphoid cancer.[67,68] The cat has the highest incidence of hematopoietic neoplasms of domestic animals, and the prevalence of lymphoma ranges from 44 to 200 cases per 100,000 cats, six times the rate of this disease in humans.[31] Twenty years ago, 70% of lymphomas in cats were believed to be caused by FeLV.

Some cancers are more commonly associated with FeLV infection than others. Large, granular lymphoma and globular leukocyte tumors usually test negative for FeLV,[69,70] whereas 70% to 90% of cats with nonlymphoid hematopoietic neoplasia (myeloproliferative disease) test positive for FeLV.[31] The percentage of lymphomas that test positive for FeLV also varies, depending on the anatomic location of the tumor.[71-73] Cats with spinal, mediastinal, ocular, and renal lymphoma frequently test positive for FeLV (more than 70%).[74] Extranodal sites, such as lymphomas of the nasal cavity and the alimentary form of lymphoma, frequently test negative for FeLV infection.[31] Over the past 20 years, the multicentric FeLV-positive form has declined in young cats, and the FeLV-negative alimentary form in older cats has increased.[75-77] Although the alimentary form most often is FeLV negative, as assessed by IFA and ELISA testing, some of these lesions have been shown by PCR to be FeLV positive, which suggests that the disease may be related to previous FeLV exposure.

Although not all lymphomas are caused by FeLV, the relative risk of developing lymphoma is 62 times higher in FeLV-positive cats, and cats that are FeLV negative but that have had previous exposure to FeLV have a fortyfold increase in the risk of developing lymphoma.[78] Most spontaneous lymphomas of cats that test positive for FeLV arise from T cells, whereas FeLV-negative lymphoma frequently is of alimentary or B-cell origin.[79,80] The time from infection to tumor development varies and may depend on the age at which the cat is infected or on other factors, such as strain, anatomic location, and viral subgroup.[31] The range from the time of experimental infection to tumor production is 1 to 23 months (mean, 5.3 months).[81,82] The younger the cat when infected with FeLV, the shorter the time to the development of neoplastic disease. Some cats infected with FeLV die of immunosuppressive disease before tumors have a chance to develop.

Treatment of FeLV Infections

Although no effective treatment exists to eliminate established FeLV infection in cats, a variety of antiviral and biologic response modifiers have been used to manage retroviral infections in cats and humans. The mainstay of therapy for cats infected with FeLV or other retroviruses is supportive care.[83-85] Maintaining hydration and nutritional status not only prolongs life but also enhances the patient's quality of life. The cat should be kept in a humid environment to reduce the chance of water loss. Appetite stimulants and placement of gastrostomy tubes may facilitate nutritional therapy. The cat's requirement for B vitamins is eight times that of the dog, and dietary concentrations must be maintained to maintain appetite. Semimoist cat foods often contain propylene glycol, which can shorten red blood cell survival. These foods, although often quite palatable,

should not be used for the nutritional management of cats infected with FeLV.[84] Many cats with FeLV are anemic, but administering erythropoietin is not helpful, because endogenous erythropoietin levels usually are 20 times normal.[86]

Biologic Response Modifiers

A variety of biologic response modifiers (BRMs) has been used in cats infected with FeLV,[87-95] but none has shown benefit in controlled trials. A few of the most popular are discussed here.

Interferons have been studied extensively for the management of FeLV infection, but the results have been mixed. In one study, oral and parenteral doses of either human recombinant interferon-α or bovine interferon failed to alter the viremia or result in clinical improvement. However, some uncontrolled studies have shown improvement in the clinical status of cats treated with oral interferon-α.[94] Controlled trials are needed to establish the true efficacy of these products. Orally administered interferon probably is inactivated by gastric acid in the stomach. Parenterally administered interferons from other species (i.e., bovine and human) are likely to have temporary activity because of the production of neutralizing antibodies.

Carrisyn (Acemannan) is a biologic response modifier designed to enhance macrophage phagocytosis and cell killing. Viremic cats treated with carrisyn have been reported to improve clinically; however, the studies reporting these results have not been well controlled, and the observed benefit may be due to the natural waxing and waning clinical course commonly observed in FeLV-positive cats.[95]

The apparent positive effect of many of the BRMs, which in fact may be due to the anabolic effect observed with some of these cytokines, is thought to be based on endorphin release rather than a direct effect on the viral infection.

Reverse Transcriptase Inhibitors

Drugs that inhibit RT and retrovirus integration into the host cell have been evaluated for their potential use in the treatment of FeLV-positive cats. The drugs evaluated include suramin (a polyionic dye used to treat filariasis in humans), nucleoside analogs (AZT, DDC, DDA, and PNEA), glucose homopolymers, dextran sulfate, phosphonate, and others.[96-99] More detailed descriptions of these therapies are provided elsewhere.[96-99] In general, most of these agents have shown efficacy *in vitro* against FeLV, the human immunodeficiency virus (HIV), and in some cases, FIV. Most of these drugs result in some reduction in viremia *in vivo*, but none are capable of reversing established viremia, although some may prevent viremia if administered prophylactically. Most of these drugs cause significant toxicities at the dosages needed to produce antiviral effects and therefore have

not gained popularity in clinical practice. AZT (zidovudine) is the most widely studied RT inhibitor.[100] AZT inhibits FeLV reverse transcriptase when administered at a dosage of 10 to 20 mg/kg in daily divided doses. AZT prevents viremia if given within 72 hours of exposure to FeLV. The antiviral effects of AZT appear to be synergistic with interferon.[101,102] Reversal of established experimental FeLV viremia through adoption transfer of lectin/interleukin-2–activated lymphocytes, interferon-α, and AZT has been reported.

Prevention and Control

The most effective means of preventing FeLV infection is to eliminate contact with viremic cats. The test and removal program is the most effective means of controlling FeLV in multiple-cat households.[103] The program consists of closing the household or cattery to new cats, testing the remaining cats every 3 months, and removing all animals that test positive. When all cats test negative for two consecutive sessions, the facility is determined to be FeLV free. New cats may enter the household or facility only after a 3-month quarantine and two negative FeLV tests. The test and removal system has been shown to reduce the incidence of FeLV in a variety of settings and geographic locations.[103]

Prevention by Vaccination

Vaccinations help control or eliminate many infectious diseases in veterinary medicine. The first commercial FeLV vaccine was introduced in 1985. Since then, seven FeLV vaccine products from six companies have been licensed for sale to veterinarians in the United States. Despite the fact that FeLV vaccines have been available for a decade, a survey of U.S. veterinary teaching hospitals in 1991 found that only two of the 22 teaching hospitals considered FeLV vaccination to be part of their routine feline preventative medicine program.[104] The principal concern has been the perceived lack of efficacy of the FeLV vaccines. Some studies of available vaccines have reported efficacies ranging from 0 to 100%.[105] In addition to efficacy questions, it has been established that soft tissue sarcomas may develop after FeLV and rabies vaccination.[106]

FeLV vaccination issues are discussed elsewhere.[66] However, several comments regarding FeLV vaccines should help practitioners decide whether to use FeLV vaccines in their practice. FeLV vaccines may contain two or three subgroup antigens. Because only subgroup A is transmitted contagiously between cats, vaccines need only to contain subgroup A. Their primary means of protecting against tumor development is preventing persistent viremia. If a vaccine protects against persistent FeLV infection, it need not contain FOCMA. The value of FOCMA in FeLV vaccines has yet to be proven. Vaccines should protect against a variety of strains of subgroup A, and none of the available vaccines contain

more than one strain of subgroup A. Differences in published comparative studies of vaccine efficacy probably are related to differences in vaccine strains and to the challenge strains used. Vaccines that contain adjuvants enhance immunity but at the expense of producing local inflammatory reactions at the injection site.[107] These local reactions may lead to the development of soft tissue sarcomas. However, the development of soft tissue sarcomas after vaccination, either with rabies or FeLV vaccines, is thought to occur in only 1 in 1000 to 1 in 10,000 vaccinates.[106] Nonadjuvanted FeLV vaccines have shown little or no inflammatory reaction 21 days after administration.[107] A canarypox-vectored FeLV nonadjuvanted vaccine recently has become available that stimulates both cellular and humoral immunity without significant injection site inflammation. Clinical discretion should be used when recommending FeLV vaccines.

The American Association of Feline Practitioners (AAFP) does not consider FeLV vaccine a core vaccine, and only cats at significant risk should be vaccinated. Cats under 12 weeks of age have an 85% chance of becoming persistently infected, whereas cats over 6 months of age have only a 15% chance of becoming persistently infected after challenge. Age-acquired immunity of adult cats is associated with improved macrophage function and reduced viral receptors on target tissues. Annual vaccination of adult cats also appears to be a questionable practice, given age-acquired immunity and the risk of vaccine-associated sarcomas.

Feline Sarcoma Virus

Feline sarcoma viruses are true hybrids that result from the rare recombination of FeLV DNA provirus with cat proto-oncogenes. Cats have at least 30 proto-oncogenes.[31,108,109] Proto-oncogenes have many biologic functions; when they are altered and activated inappropriately, they are called *oncogenes*, which can play a key role in the development of cancerous phenotypes (see Chapter 2). Proto-oncogenes can be activated by mutations that produce chromosomal translocations, such as those that may be associated with inflammation and vaccine-associated sarcomas, or by incorporation into a retrovirus, such as FeLV.[108,109,110] When FeLV-derived DNA inserts near a proto-oncogene and takes up the proto-oncogene into the FeLV provirus, formation of FeSV results. In the process, part of the FeLV GAG gene, most of the FeLV envelope gene, and all of the pole genes are lost.[109] The loss of these vital components makes FeSV dependent on FeLV as a helper virus for replication. Cats that have FeSV always test FeLV positive. Because several different recombinations may recur with several different proto-oncogenes, each recombination is a unique event, and each isolate is distinct.[110] Despite this phenotypic heterogeneity, the recombinations transform fibroblasts, and all produce fibrosarcomas.

Natural transmission of FeSV between cats has not been described and, as with other FeLV recombinants (e.g., FeLV-B), transmission of the recombinant product is not thought to occur in nature. Some cats are capable of rejecting transformed cells and producing FOCMA antibody.[85,86] FOCMA is important in the experimental response of cats to FeSV, because it has been associated with tumor regression and failure to develop tumors.[111,112] Cats that fail to develop antibodies against FOCMA die quickly of fast-growing sarcomas.[113]

Clinical Features of FeSV and Induced Fibrosarcomas

Only 2% of fibrosarcomas of cats are virally induced.[37] In contrast to the solitary, slow-growing, non-virally-induced sarcomas seen in older cats, FeSV-induced tumors are multicentric and are found most frequently in young cats.[114] They are characterized by rapid growth, including doubling times as short as 12 to 72 hours.[31] This rapid growth often is accompanied by superficial ulceration. Lesions frequently occur at sites of previous bite wounds.[31] Metastasis to the lungs or other organs occurs with approximately 30% of virally induced fibrosarcomas in cats. Hypercalcemia was observed in association with multicentric fibrosarcomas one cat with.[31] Virally induced fibrosarcomas are always FeLV positive; this helps differentiate them from vaccine-associated sarcomas, which have growth characteristics similar to those of virally induced tumors. Cats with multicentric FeSV-induced tumors have a very poor prognosis. Doxorubicin, vincristine, vinblastine, lomustine (CCNU), and radiotherapy have been used to treat vaccine-associated sarcomas in the cat.[115] Although radiotherapy often is used in combination with surgery, recurrence both within and outside the treatment fields is common.

Feline Immunodeficiency Virus

FIV, which is classified as a retrovirus in the subfamily Lentivirinae, is distinct from other retroviruses that infect cats. Like other retroviruses, FIV is an enveloped, single-stranded RNA virus in which the RNA is copied into the DNA in the infected host by RT in the virus.

The nucleotide sequence of several FIV isolates has been determined, and genetic homology falls between 36% and 97%. Despite this homology, significant differences in pathogenicity and infectivity exists between FIV strains.[116,117] Although lentiviruses are known to infect wild felids, they are antigenically distinct from domestic cat isolates; they also are well adapted to their host and seldom cause clinical disease.

Transmission

FIV is present in all bodily fluids of infected cats, similar to FeLV, but at much lower concentrations. FIV is mainly cell associated and is present in relatively low concentrations in the blood, although high amounts can be found in the saliva.[118,119] FIV is not thought to be very infectious and is mainly transmitted through biting during cat fights.[120,121]

FIV-Associated Neoplasms

The prevalence of neoplasms in FIV-positive cats ranges from 1% to 62%.[78,122,123] Lymphomas and myeloid tumors (myelogenous leukemia, myeloproliferative disease) and a few carcinomas and sarcomas are the neoplasms most commonly linked to FIV infection. One study found that cats infected with FIV and FeLV are 5.6 times more likely to develop lymphoma or leukemia than if they had been infected with either virus alone. Cats with combined infections had a 77.3% greater likelihood of developing lymphoma or leukemia than noninfected cats.[78] Lymphoreticular neoplasms have been linked to HIV infection in humans and simian immunodeficiency virus (SIV) infection in nonhuman primates. In contrast to FeLV-associated lymphomas, FIV-associated lymphomas most often develop in extranodal sites and occur in older cats (mean age, 8.7 years).[78] Myeloproliferative disease also has been observed in cats naturally and in cats experimentally infected with FIV.[78,124,125]

Although lentiviruses such as FIV have not been thought to be oncogenic in themselves, they are markedly immunosuppressive and affect normal immunosurveillance of cancerous cells. FIV-positive cats with lymphoma have extremely low CD4 lymphocyte counts.[115] Squamous cell carcinomas of the skin have been linked to FIV infection in two geographic areas, California and Colorado, but this association is believed to be due to a co-risk behavior (outdoor cats) rather than to any direct viral contribution to tumor development.[126,127] Other reports have linked FIV infection to oral squamous cell carcinoma, mammary carcinoma, fibrosarcoma, myeloproliferative disease, and histiocytic mast cell disease.[122,128,129] The nature of these associations awaits further investigation.

Treatment

The same treatment considerations in the management of cats with FeLV can be applied to the treatment of FIV-positive cats. The most widely applied treatments have been the RT inhibitors and interferon (see the earlier discussion on the treatment of FeLV). As in the treatment of FeLV, FIV-positive cats remain positive despite these therapies. A single inactivated FIV vaccine has been licensed for use in domestic cats. However, this is an adjuvanted vaccine, and it may be associated with an increased risk of vaccine-associated sarcoma. The primary concern with the vaccine is that it generates antibodies that cross-react with the currently recommended antibody-based diagnostic test for FIV infection. PCR-based tests are not currently considered

reliable for diagnosis of FIV, and antibody-based testing remains the gold standard. It is important to note that the AAFP does not recommend the use of an FIV vaccine in domestic cats.

COMPARATIVE ASPECTS

The association between human viruses and certain cancers has been established on the basis of epidemiologic, clinical, and molecular studies.[1] Human T-cell leukemia virus (HTLV-1) has been linked to adult T-cell leukemia. The human papillomavirus (HPV) is associated with cervical cancer. The Epstein-Barr virus (EBV) is related to the development of Burkitt's lymphoma and nasopharyngeal carcinoma, and human hepatitis B and C viruses are associated with hepatocellular carcinoma. The human herpes virus (HHV) is implicated in the development of Kaposi's sarcoma.

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CHAPTER 2

Tumor Biology and Metastasis

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Multicellular organisms organize as specialized societies that cooperate to promote survival of the organism. In this model, cell division, proliferation, and differentiation are strictly controlled to create a balance between normal cell birth and natural cell death.[1] Derangement of these normal homeostatic mechanisms can lead to uncontrolled proliferation or loss of appropriate death, leading to a normal cell taking on a malignant phenotype.

Cancer in animals is well documented throughout history but has taken on significance over the past hundred years for a number of reasons. Studies on chicken, feline, and bovine retroviruses have made significant contributions to our overall understanding of carcinogenesis through the discovery of oncogenes and tumor suppressor genes.[2] Further contributions to our understanding of viral oncogenesis have come from studies of the DNA papilloma viruses in cattle and horses, complementing research into cervical cancer in women.[3-10] This translational cancer research has paved the way for the development of programs of cancer research in comparative medicine that holds benefits for both humans and the veterinary species. In this chapter, we summarize the current understanding of the molecular mechanisms of cancer development and metastasis. To appreciate these complex pathways, we first describe the normal mechanisms of cell division and proliferation.

NORMAL CELL DIVISION

Within an animal, all cells are subject to wear and tear, making cellular reproduction a necessity for maintenance of the individual. Reproduction of the gametes occurs by the process of meiosis, whereas reproduction of somatic cells involves two sequential phases known as mitosis and cytokinesis. Mitosis is nuclear division, and cytokinesis involves the division of the cytoplasm, the two occurring in close succession. Nuclear division is preceded by a doubling of the genetic material within the cell during a period known as *interphase*. As well as duplication of the chromosomes, this period is characterized by marked cellular activity in terms of RNA, protein, and lipid production. The alternation between mitosis and interphase in all tissues is often referred to as the **cell cycle**. The phases of the cell cycle are shown in Figure 2-1.

Interphase (G1, G2, and S phases) is the longest phase of the cell cycle. During interphase, the chromatin are very long and slender but, as interphase progresses, they progressively shorten and thicken. The first phase of mitosis is referred to as *prophase* and marks the first appearance of the chromosomes. As mitosis progresses, the chromosomes appear as two identical sister chromatids joined at the centromere. As the nuclear membrane disappears, spindle fibers form and radiate from the two centrioles, each located at opposite poles of the cell. The spindle fibers pull the chromosomes to opposite sides of the cell.

During *metaphase*, the spindle fibers pull the centromeres of the chromosomes, which become aligned to the middle of the spindle, often referred to as the equatorial plate. During *anaphase*, the centromeres split and the sister chromatids are pulled apart by the contraction of the spindle fibers. The final stage of cell division is telophase and is characterized by the formation of a nuclear membrane around each group of chromosomes followed by cytokinesis or separation of the cytoplasm to produce two identical diploid cells. Progression through the cell cycle lasts for around 12 to 24 hours.

The Cell Cycle

The cell cycle is made up of four phases: M phase, S phase, G1, and G2 (see Figure 2-1). Nonproliferating cells are usually arrested between the M (mitosis) and S (DNA synthesis) phases and are referred to as G0 (Growth 0) cells. The majority of cells in normal tissues are in G0. Cells are stimulated to enter the cell cycle in response to external factors including growth factors and cell adhesion molecules. During the G1 (Growth 1) phase of the cell cycle, cells are responsive to mitogenic signals. Once the cell has progressed through a defined point (the restriction point; R) in the G1 phase, then cell cycle transitions become autonomous.
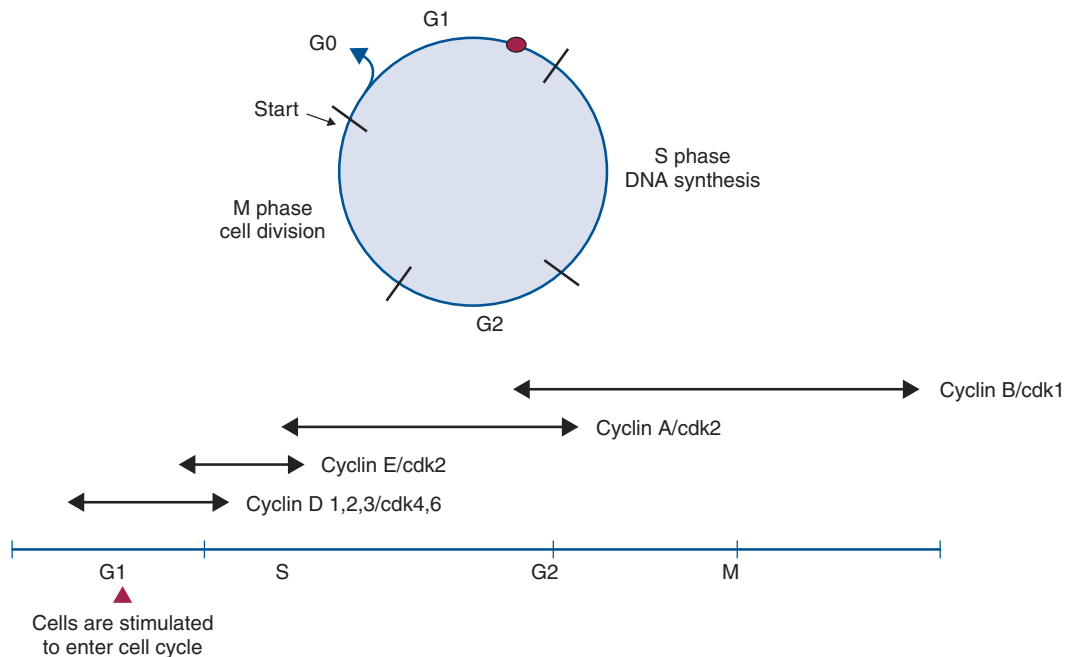
Figure 2-1

The Cell Cycle and its Control. The cell cycle is divided into four phases (G1, S, G2, and M) and G0, which represents cycle-arrested cells. Cells are stimulated to enter the cell cycle in response to external factors, including growth factors and cell adhesion molecules. During G1, cells are responsive to mitogenic signals. Once the cell cycle has traversed the restriction point (R) in the G1 phase, the cell cycle transitions become autonomous. Progression through the cell cycle is mediated by the sequential activation and inactivation of the cyclin-dependent kinases (CDKs). Control of CDK activity is through their interaction with specific cyclins (D, E, A, B) and with specific cyclin-dependent kinase inhibitors.

Progression through the cell cycle is mediated by the sequential activation and inactivation of a class of proteins called cyclin-dependent kinases (CDKs) (reviewed in[11,12]). The CDKs regulate a series of checkpoints that mediate mitogenic and inhibitory signals. CDKs consist of an inactive conserved catalytic core and are regulated at three levels:

1. CDK kinase activity requires the association with regulatory subunits known as cyclins (Cyclins A-H). The levels of CDKs remains constant throughout the cell cycle; however, the concentration of cyclins varies in a phase specific manner during the cell cycle. Thus, the periodic synthesis and destruction of cyclins provide the primary level of cell cycle control (see Figure 2-1).

2. The activity of cyclin/CDK complexes is also regulated by phosphorylation. Activation of CDK/cyclin complexes requires phosphorylation by CDK activating kinases (CAK) and the phosphorylation at threonine and serine residues suppresses activity.

3. CDKs are also tightly regulated by a class of inhibitory proteins known as cyclin-dependant kinase inhibitors (CDKI). CDKIs can block G1/S progression by binding CDKs/cyclin complexes and can be classified into two groups:

   a. INK4A family (p15^{INK4b}, p16^{INK4a}, p18^{INK4c}, and p19^{INK4d}). These act primarily on cdk4 and cdk6 complexes and prevent the association with cyclin D (See the text that follows).

   b. CIP/KIP family (p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}). These are less specific and can inactivate various cyclin/CDK complexes.

Following mitogenic stimulation of the cell cycle, the first class of proteins induced in G1 are the class D cyclins, which in turn activate CDK4 and CDK6. Cyclin D-CDK complexes cause phosphorylation of the substrate retinoblastoma protein (Rb), which results in dissociation of the transcription factor E2F from the Rb protein. The phosphorylation status of Rb plays a critical role in regulating G1 progression, and Rb is the molecular device that serves as the R point switch. This R point acts as a "go-no-go" point in the G1 phase of the cell cycle. Cells that pass through this R point will progress into the S phase of the cell cycle. At the R point, phosphorylated

Rb releases E2F enabling transcription of numerous E2 responsive genes involved in DNA synthesis, whereas unphosphorylated pRB remains associated with E2F and inhibits cell cycle progression. During G1 progression, activation of E2F also leads to induction of cyclin E. Cyclin E associates with CDK2 and the cyclin E-CDK2 complex maintains Rb in the phosphorylated state and is essential for cells to enter the S phase of cell cycle. At the G1/S phase transition, E2F induces cyclin A. During early S phase, cyclin D and E are degraded and cyclin A associates with CDK2 and CDK1, and this kinase activity is essential for entry into S phase, completion of S phase, and entry into M phase. Mitosis is regulated by CDK1 in association with cyclins A and B causing phosphorylation of cytoskeletal proteins including histones and lamins.

CELLULAR RESPONSES TO DNA DAMAGE

When normal cells are subjected to stress signals (i.e., radiation), DNA damage, or oxygen depletion, they are able to undergo **cell cycle arrest** in G1, S, and G2 or enter programmed cell death (**apoptosis**) or both. Within cells, surveillance systems that are active at the cell cycle checkpoints operate to recognize and respond to DNA damage. Cell cycle checkpoints occur in the G1 phase in response to DNA damage, during S phase to monitor the quality of DNA replication and the occurrence of DNA damage, and during the G2/M phase to examine the status of the spindle. The tumor suppressor protein, p53, is one of the most well studied proteins that regulates checkpoints in response to DNA damage. The p53 protein plays an important role in maintaining genomic stability and forms part of a stress response pathway to various exogenous and endogenous DNA damage signals including gamma irradiation, UV irradiation, chemicals, and oxidative stress.

The p53 Protein Functions as a Genomic Guardian

The p53 response to stress, as just described, may be mediated by DNA dependant protein kinase (DNA-PK) or by the ATM kinase and leads to phosphorylation of the N terminus of p53. In the absence of DNA damage, p53 is unphosphorylated and is short lived; however, in the presence of DNA damage, p53 becomes stabilized as a result of phosphorylation. In this active state, p53 can then function as a transcriptional regulator binding to sequences and transactivating a number of genes including the CDK inhibitor p21.[13,14] The p21 protein has a high affinity for G1 CDK/cyclin complexes and inhibits kinase activity, thereby arresting cells in G1. By holding cells in G1, the replication of damaged DNA is prevented and the cellular DNA repair machinery has the opportunity to repair damage prior to reentering the active growth cycle (Figure 2-2).

The cellular levels of p53 protein are regulated by the product of another gene MDM2 (mouse double minute 2 oncogene).[15] The principal role of MDM2 is to act as a negative regulator of p53 function. One mechanism for MDM2 to downregulate p53 is to target p53 for degradation, via a ubiquitin proteosome pathway. MDM2 and p53 also regulate each other at the transcriptional level. MDM2 is a transcriptional target of p53, and its expression is induced by the binding of p53 to an internal promoter within the mdm2 gene. MDM2 can in turn bind to a domain within the amino terminus of p53, thereby inhibiting the transcriptional activity and G_1 arrest function of p53 by masking access to the transcriptional machinery (see Figure 2-2).[16]

Cell Death

Where cells are damaged and are unable to repair DNA, the cell can be directed into programmed death or apoptosis. In contrast to necrosis, apoptosis is a distinct type of cell death characterized by "programmed" self-destruction of cells that occurs in disease states as well as part of normal physiological cell turnover. Whereas necrosis is characterized by swelling of the cell and lysis, in apoptosis there is cellular and nuclear shrinkage followed by fragmentation and subsequent phagocytosis. The molecular mechanisms involved in apoptosis are shown in Figure 2-3 and reviewed in Wyllie et al. and Evan and Littlewood.[17,18] Apoptosis provides a controlled mechanism for eliminating cells that are irreversibly damaged and involves an ATP-dependent activation of cellular pathways, which moves calcium from the endoplasmic reticulum to the cytoplasm and activation of endonucleases by either intrinsic (often involving mitochondrial membrane fluxes) or extrinsic signals. Some of these pathways are mediated through caspases. A wide variety of signals can initiate an apoptotic response, including Fas ligand (CD95 or FasL) and its interaction with the Fas receptor, tumor necrosis factor, and certain oncogenes. The Fas and TNF receptors are members of the death receptor family. These are transmembrane proteins with cysteine rich extracellular domains and intracellular regions that share a common structure termed the "death domain." The pro-apoptotic ligands for these receptors are homotrimeric peptides that are either soluble or expressed at the surface of the adjacent cell. Ligand-induced receptor clustering promotes the binding of a soluble cytosolic adapter protein called Fas-associated death domain (FADD), which itself contains a death domain as well as a caspase binding site, to the clustered death domains of the receptors. This leads to activation of caspase 8 and downstream activation of effector caspases for apoptosis.

Figure 2-2

p53 is involved in cell cycle control. The p53 response to stress may be mediated by DNA dependant protein kinase (DNA-PK) or by the ATM kinase and leads to phosphorylated stable p53, which can then function as a transcriptional regulator binding to sequences and transactivating a number of genes including p21 and Bax. Consequently, the cell cycle is arrested or the cell undergoes programmed cell death (apoptosis). This is a defense mechanism that allows for repair or death (if the cell is irreversibly damaged). The cellular levels of p53 protein are regulated by the product of another gene, mouse double dinute 2 oncogene (MDM2), which acts as a negative regulator of p53 function. One mechanism for MDM2 to downregulate p53 is to target p53 for degradation via a ubiquitin proteosome pathway. Alternatively, MDM2 can also control p53 function by suppressing p53 transcriptional activity.

SUMMARY

The cell cycle ensures a regulated process of DNA replication before cell division occurs. The cell responds to growth and environmental signals through the cell cycle. Components of the cell cycle that have a stimulatory effect include the cyclins and the cyclin dependent kinases. The negative influences come from a series of checkpoints that respond to external stimuli. These include tumor suppressor genes such as p53 and Rb, and also genes involved in DNA repair. The process of DNA replication is also subject to the introduction of errors, and this is closely monitored by a class of enzymes called DNA repair enzymes. Consequently, there are a number of safeguards within the cell cycle to ensure that normal cells are produced during division and that the DNA is accurately replicated. In the next section, we describe how these systems are overcome to produce a malignant cancer cell.

FROM NORMAL CELL TO CANCER CELL

It is difficult to define a cancer cell in absolute terms. Tumors are usually phenotypically recognized by the fact that their cells show abnormal growth patterns and are no longer under control. Although the range of mechanisms involved in the development of tumors and the spectrum of tissues from which tumors are
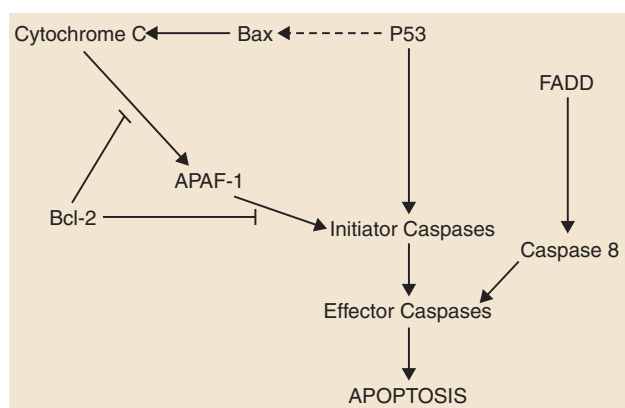
Figure 2-3

The mediators of apoptosis. A wide variety of signals can initiate an apoptotic response, including Fas ligand (CD95 or FasL) and its interaction with the Fas receptor, tumor necrosis factor and its receptor interaction, and certain oncogenes. The Fas and TNF receptors are members of the death receptor family. These are transmembrane proteins with cysteine-rich extracellular domains and intracellular regions that share a common structure termed the *death domain*. The proapoptotic ligands for these receptors are homotrimeric peptides that are either soluble or expressed at the surface of the adjacent cell. Ligand-induced receptor clustering promotes the binding of a soluble cytosolic adapter protein called Fas-associated death domain (FADD), which itself contains a death domain as well as a caspase binding site, to the clustered death domains of the receptors. This leads to activation of caspase 8 and downstream activation of effector caspases for apoptosis.

derived are diverse, they can be classified into three broad types.

1. *Benign tumors*. Broadly speaking, these tumors arise in any of the tissues of the body and grow locally. Their clinical significance results from an ability to cause local pressure, cause obstruction, or form a space-occupying lesion such as a "benign" brain tumor. Benign tumors do not metastasize.
2. *In situ tumors*. These are often small tumors that arise in the epithelium. Histologically, the lesion appears to contain cancer cells, but the tumor remains in the epithelial layer and does not invade the basement membrane or the supporting mesenchyme. A typical example of this is a preinvasive squamous cell carcinoma affecting the nasal planum of cats and is often referred to as Bowen's disease or Bowenoid carcinoma *in situ*.
3. *Cancer*. This refers to a malignant tumor that has the capacity for both local invasion and distant spread by the process of metastasis.

Multistep Carcinogenesis

Cancer is the phenotypic end result of a series of genetic and nongenetic events that may take place over a long period of time. The corollary of this statement is that there are very few single causes of cancer. Accordingly, the application of a cancer-producing agent (carcinogen) to a tissue does not lead to the immediate production of a cancer cell. A carcinogenic agent will often result in the *initiation* step of the carcinogenic cascade. There follows a period of tumor *promotion* that may be caused by the same initiating agent or by other substances such as normal growth promoters or hormones. The *initiating* step is a rapid step and affects the genetic material of the cell. If the cell does not repair this damage, then *promoting* factors may progress the cell further down the carcinogenic cascade toward a malignant phenotype. In contrast to initiation, progression may be a slow process and may not even manifest in the lifetime of the animal. Each stage of multistep carcinogenesis reflects genetic changes in the cell with a selection advantage that drives the progression toward a highly malignant cell. The age-dependent incidence of cancer suggests a requirement for between four and seven rate limiting, stochastic events to produce the malignant phenotype.[19]

These sequential events in tumor formation are a consequence of changes at the genetic level. Since the early 1980s, cancer research has generated a rich and complex body of information revealing that cancer is a disease involving dynamic changes in the genome. Seminal to our understanding of cancer biology has been the discovery of the so-called cancer genes or *oncogenes* and *tumor suppressor genes*. Mutations that produce oncogenes with dominant gain of function and tumor suppressor genes with recessive loss of function have been identified through their alteration in human and animal cancer cells and by their elicitation of cancer phenotypes in experimental models.

Oncogenes

The RNA tumor viruses (retroviruses) provided the first evidence that genetic factors play a role in the development of cancer. The initial observation came in 1910 when Rous demonstrated that a filterable agent (later classified as a retrovirus and termed avian leukosis virus) was capable of producing lymphoid tumors in chickens.[20] Retroviruses have three core genes (gag, pol, and env) and an additional gene that gives the virus the ability to transform cells. Retroviral sequences that are responsible for transforming properties are called **viral oncogenes (v-onc)**. The names of these genes are derived from the tumors in which they were first described (e.g., v-ras from rat sarcoma virus).

Viral oncogenes were subsequently shown to have cellular homologues called **cellular oncogenes (c-onc)**.

The term proto-oncogene was later used to describe cellular oncogenes that do not have transforming potential to form tumors in their native state but can be altered to lead to malignancy.[21] Most proto-oncogenes are key genes involved in the control of cell growth and proliferation, and their roles are complex. For simplicity, their sites and modes of action in the normal cell can be divided as follows (Table 2-1):

- Growth factors
- Growth factor receptors
- Protein kinases
- Signal transducers
- Nuclear proteins and transcription factors

Growth Factors

These are molecules that act on the cell via cell surface receptors. Their contribution to carcinogenesis may be through excessive production of the growth factor or where a growth factor is expressed in a cell that does not normally produce the growth factor or in a cell that also expresses the same growth factor receptor (autocrine activation).

Growth Factor Receptors

Several proto-oncogene–derived proteins form part of cell surface receptors for growth factors. Signal transduction through a growth factor receptor (either ligand dependent or independent) may be one of the earliest mitogenic signals received by cells. Their role in carcinogenesis may be through structural alterations in these proteins.

Protein Kinases

Protein kinases are associated with the inner surface of the plasma membrane and are involved in signal transduction following growth factor receptor activation. Structural changes in these genes and proteins lead to increased kinase activity that can have profound effects on signal transduction pathways.

TABLE 2-1	Metastasis Promoting Gene/Protein	
Oncogene	**Name**	**Abbreviation**
Growth factors	Platelet derived growth factor	PDGF
	Epidermal growth factor	EGF
	Insulin-like growth factor-1	ILGF-1
	Vascular endothelial growth factor	VEGF
	Transforming growth factor-β	TGF-β
	Interleukin-2	IL-2
Growth factor receptors	PDGF-receptor	PDGF-R
	EGFR-receptor	erbB-1
	ILGF-1 receptor	ILGF-R
	VEGF-receptor	VEGFR
	IL-2 receptor	IL-2R
	Hepatocyte growth factor receptor	met
	Heregulin receptor	neu/erbB-2
	Stem cell factor receptor	kit
Protein kinases	Tyrosine Kinase	bcr-abl
	Tyrosine Kinase	src
	Serine-threonine kinase	raf/mil
	Serine threonine kinase	mos
G-protein signal transducers	GTPase	H-*ras*
	GTPase	K-*ras*
	GTPase	N-*ras*
Nuclear proteins	Transcription factor	ets
	Transcription factor	fos
	Transcription factor	jun
	Transcription factor	myb
	Transcription factor	myc
	Transcription factor	rel

Signal Transduction

The series of events by which mitogenic signals are transduced from the activated growth factor receptor to the nucleus of the cell is collectively referred to as signal transduction. Intimate to some signaling pathways are the second messengers such as guanosine triphosphate (GTP) and proteins that bind GTP (G-proteins). During signal transduction, GTP is converted to guanosine diphosphate (GDP) by the GTPase activity of G-proteins. A group of proto-oncogenes called Ras encodes proteins with GTPase and GTP binding activity and, in the normal cell, helps to modulate cellular proliferation. Mutations in the Ras proto-oncogene can contribute to uncontrolled cellular proliferation. A more complete review of signal transduction and methods of therapeutically manipulating the process is presented in Chapter 14, Section B.

Nuclear Proteins and Transcription Factors

These proto-oncogenes encode proteins that control gene expression. These genes may have a role in cellular proliferation, and changes in transcription factor activity may contribute to the development of the malignant genotype.

Mechanisms by Which Oncogenes Become Activated

The advent of recombinant DNA technology has allowed scientists to unravel a number of mechanisms by which the normal products of proto-oncogenes can be disrupted to produce uncontrolled cell division. The conversion of a proto-oncogene to an oncogene is a result of somatic events in the genetic material of the target tissue. The activated allele of the oncogene dominates the wild-type allele and results in a **dominant gain of function.** This means that only one allele has to be affected to obtain phenotypic change and is in contrast to tumor suppressor genes where both alleles have to be lost for phenotypic change. Mechanisms of oncogene activation are outlined next and are shown in Figure 2-4 and reviewed in several studies.[22–28a]

- *Chromosomal translocation.* Where proto-oncogenes are translocated within the genome (i.e., from one chromosome to another), their function can be greatly altered. The classical example is the chromosomal breakpoint that produces the Philadelphia chromosome found in chronic granulocytic leukemia in humans. This involves the translocation of the c-abl oncogene on chromosome 9 to a gene on chromosome 22 (bcr). The point at which two genes come together is referred to as a *chromosomal breakpoint.* The BCR/ABL hybrid gene produces a novel transcript whose protein product has elevated tyrosine kinase activity and can contribute to uncontrolled cellular proliferation. Transgenic mice for this chimeric gene develop lymphoblastic leukemia and lymphoma. This gene product has

Figure 2-4

Methods of oncogene activation. Oncogenes can be activated by **(A)** Chromosomal rearrangements (e.g., BCR/ABL induced leukemia resulting from a translocation of chromosome 9 and 22, leading to the so called "Philadelphia chromosome"), **(B)** gene amplification (e.g., amplification or abnormal duplication of MDM2 in some sarcomas), **(C)** point mutations (e.g., changes in nucleotide sequence which alters protein production), or by **(D)** viral insertions (e.g., the insertion of FeLV at the myc locus in FeLV induced lymphomas).
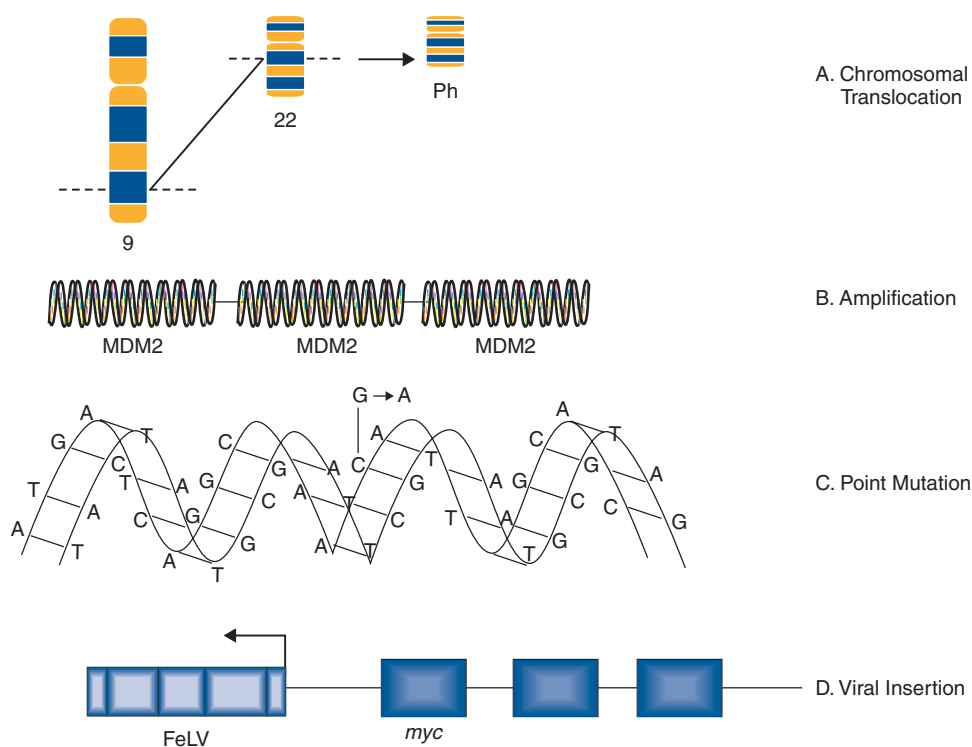
become a target for tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia (CML) in people (See Chapter 14, Section B).

- *Gene amplification.* Quantification of gene copy number is possible by southern hybridization and subsequent densitometric analysis comparing a gene's expression to that of "house-keeping" genes. Amplification of oncogenes can occur in a number of tumor types and has been demonstrated in childhood neuroblastoma, where the myc proto-oncogene (nuclear transcription factor) is amplified up to 300 times. Gene amplification is considered the most common mechanism of proto-oncogene activation. A further example is the MDM2 proto-oncogene, which has been identified in dogs and horses and has been shown to be amplified in a proportion of canine soft-tissue sarcomas.

- *Point mutations.* These are single base changes in the DNA sequence of proto-oncogenes leading to the production of abnormal proteins and can arise through the actions of ionizing radiation or chemical carcinogens. A mutation in a proto-oncogene or the transcriptional machinery that controls its expression may disrupt homeostasis and result in sustained proliferation signals or a failure to respond to negative feedback signals. A classical example is the Ras proto-oncogene where point mutations are a consistent finding in a number of human tumors. K-ras mutations have also been identified in canine lung tumors and pancreatic tumors.[28b,28c] Mutations in Erb-B (epidermal growth factor receptor) gene have been shown in a number of human cancers and can lead to ligand independent activation. Similar activating mutations in the c-kit growth factor receptor have been identified and are thought to be important in the biology of high-grade mast cell tumors in dogs.[28d]

- *Viral insertions.* The discovery of oncogenes was a direct result of studies on tumor-causing viruses. In some circumstances, proto-oncogene function can be damaged by the insertion of viral elements. Very occasionally, novel retroviruses are isolated from animals that have been viremic with a leukemia virus for some time. These viruses induce tumors rapidly when inoculated into members of the species of origin and are referred to as acutely transforming oncoviruses. The prototype of the acutely transforming virus is the Rous sarcoma virus (RSV).[20] Subsequently, many more transforming oncoviruses have been isolated from animals infected with avian, feline, murine, or simian oncoviruses. These viruses are generated by a rare recombinational event between the leukemia virus with which the

animal was originally infected and a cellular proto-oncogene. In this, part of the viral genome is deleted and replaced with the cellular oncogene. The virus then becomes acutely transforming because this oncogene is now under the transcriptional control of the efficient viral promoters and infection of a cell and insertion of this continuously expressed oncogene into the cellular genome allows rapid progression toward malignancy. Evidence suggests that these acutely transforming viruses are not transmitted naturally, but all events occur in the individual animal. Because the virus has itself lost some of its own genetic material, it is defective for replication. However, they are spread throughout an animal by the provision of help from the normal leukemia virus, which provides the missing proteins in co-infected cells.

In addition to acutely transforming mechanisms, retroviruses can activate cellular oncogenes by integrating adjacent to them. A good example of this is the *myc* gene, which is frequently activated in feline T cell lymphomas.[23,24] In one mode, the virus integrates adjacent to the oncogene and transcription initiation in the viral long terminal repeat (LTR) proceeds into the adjacent oncogene producing a hybrid mRNA. In a second form, the enhancer of the virus overrides the regulation of transcription from its normal promoter.

Tumor Suppressor Genes

Changes in genes can lead to either a stimulatory or inhibitory effect on cell growth and proliferation. The stimulatory effects are provided by the proto-oncogenes described earlier. In contrast, tumor formation can result from a loss of inhibitory functions associated with another class of cellular genes called the tumor suppressor genes. The discovery of these genes began by observations of inherited cancer syndromes in children, in particular studies of retinoblastoma. In the early 1970s, epidemiological studies of both retinoblastoma and Wilm's tumor led Knudson to propose his "two hit" theory of tumorigenesis.[28e]

Retinoblastoma Forms the First Clues to the Existence of Tumor Suppressor Genes

Retinoblastoma occurs in two forms, a sporadic form and an inherited form (accounting for 40% of cases).[29] In the inherited form the mode of inheritance is autosomal dominant and accounts for about half the children afflicted with this cancer. Knudson's model required the retinoblastoma tumor cells (in either sporadic or inherited forms) acquire two separate genetic changes in the DNA before tumor development. The first or predisposing event could be inherited through the germ line (familial retinoblastoma) or it could arise *de novo* in

somatic cells (sporadic form). The second event occurs in somatic cells. Thus, in sporadic retinoblastoma, both events arose in the retinal cells. However, in familial retinoblastoma, the individual had already inherited one mutant gene and only required a second hit in the remaining normal gene in somatic cells.

Although the mode of inheritance of retinoblastoma is dominant with incomplete penetrance, at the cellular level, loss or inactivation of both alleles is required to change the cells phenotype. Generically, mutations in tumor suppressor genes behave very differently from oncogene mutations. Whereas activating oncogene mutations are dominant to wild type (they emit their proliferating signals regardless of the wild-type gene product), suppressor mutations are recessive. Mutation in one gene copy usually has no effect, as long as a reasonable amount of wild-type protein remains. Consequently, some texts refer to tumor suppressor genes as recessive oncogenes.

More recently, Knudson's hypothesis was confirmed when the Rb gene was cloned and characterized. The retinoblastoma tumor suppressor Rb is the principal member of a family of proteins that also encompass pRb2/p130 and p107. Rb function can be abrogated by point mutations, deletions, or by complex formation with viral oncoproteins such as SV40 large T antigen and adenoviral E1a protein. The function of additional proteins associated with the Rb pathway are also subjected to deregulation in human cancers including overexpression of D type cyclins, overexpression of CDK4, and downregulation of the CDKI cell cycle inhibitor p16.

While loss of cell cycle control via the Rb pathway occurs commonly in many human tumors, little is known about the role of Rb, cyclin D, CDK4, and p16 in tumors of domesticated animal.

The p53 Tumor Suppressor Gene

The p53 gene has been described as the guardian of the genome, by virtue of its ability to push cells into arrest or apoptosis depending on the degree of DNA damage. As described earlier, p53 is a gene whose product is intimately involved in cell cycle control. Its discovery by Sir David Lane marked a major milestone in cancer research and has allowed greater understanding of molecular mechanisms of cancer and identified potential targets for therapeutic intervention.[13] Failure of p53 function may ultimately result in abnormal, uncontrolled cell growth leading to tumorigenic transformation.[30–33]

The p53 gene is the most frequently inactivated gene in human neoplasia with functional loss commonly occurring through gene mutational events including nonsense, missense and splice site mutations, allelic loss, rearrangements, and deletions. However, p53 function can also be abrogated by several nonmutational mechanisms including nuclear exclusion, complex formation with a number

of viral proteins, and through overexpression of the cellular oncogene, mouse double minute –2 (MDM2).

The homologues of p53 and MDM2 have both been identified in domestic animal species, and a number of studies indicate that this gene also has a central role in the progression of cancers in animals.[34–38]

Cancer Arises through Multiple Molecular Mechanisms

The advances in our understanding of normal cell biology and the processes that lead to malignancy have increased dramatically since the 1970s. Since the 1990s we have seen that transformation of a normal cell into a malignant cell requires very few molecular, biochemical, and cellular changes that can be considered as acquired capabilities.[39] Further, despite the wide diversity of cancer types, these acquired capabilities appear to be common to all types of cancer. An optimistic view of increasing simplicity in cancer biology is further endorsed by the fact that all normal cells, irrespective of origin and phenotype, carry similar molecular machineries that regulate cell proliferation, differentiation, aging, and cell death.

We have discussed that tumorogenesis is a multistep process and that these steps reflect genetic alterations that drive the progression of a normal cell into a highly malignant cancer cell. This conclusion is supported by the finding that genomes of tumor cells are invariably altered at multiple sites. The spectrum of changes range from subtle point mutations in growth regulatory genes to obvious changes in chromosomal complement.

Cancer cells have defects in regulatory circuits that govern cellular proliferation and homeostasis. It has been suggested that the vast array of cancer genotypes is a manifestation of only six alterations in cellular physiology that collectively dictate malignant growth. These acquired characteristics can be summarized under the following headings:
- Self-sufficient growth
- Insensitivity to antigrowth signals
- Evasion of programmed cell death (apoptosis)
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

In one sense, cancer is a very common disease in animals and humans, but in fact the development of clinically relevant cancer is a rare event. When one considers the number of cells in the body, the proliferation of these cells, and the potential for malignant transformation, then the development of a single cancer is rare. This is because of the cell's own natural defenses against progression toward the malignant phenotype. Each of the acquired capabilities described earlier represent a breach in the cell's own homeostatic mechanisms that are in place to prevent the development of cancer. Next we present an overview of these traits and the strategies

by which they are acquired in cancer cells. The process of metastasis requires angiogenesis and invasion, as such the traits of sustained angiogenesis, and tissue invasion and metastasis, will be collectively reviewed under a final section on metastasis.

Self-Sufficiency in Growth Signals

Normal cells require mitogenic stimuli for growth and proliferation. These signals are transmitted to the nucleus by the binding of signaling molecules to specific receptors, the diffusion of growth factors into the cell, extracellular matrix components, or cell-to-cell adhesions or interactions. As previously discussed, many oncogenes act by mimicking normal growth signals. Tumor cells do not have a dependency on external mitogenic stimuli for proliferation and sustained growth but are self-sufficient. The liberation from dependency on exogenous signals severely disrupts normal cellular homeostasis.

While the acquisition of growth signaling autonomy by cancer cells is conceptually satisfying, it is in fact too simplistic. One of the major problems of cancer research is to focus on the cancer cell in isolation. It is now apparent that we must also consider the contribution of the tumor microenvironment to the survival of cancer cells. Within normal tissues, paracrine and endocrine signals contribute greatly to growth and proliferation. Cell-to-cell growth signaling is also likely to operate in cancer cells and may be as important as some of the autonomous mechanisms of tumor growth. It has been suggested that growth signals for the proliferation of carcinoma cells are derived from the tumor stromal elements. It is therefore possible that the survival of tumor cells not only relies on the acquisition of growth signal autonomy but may also require the recruitment of local cells to provide them with growth signals.

Insensitivity to Antigrowth Signals

Within the normal cell, multiple antiproliferative signals operate to maintain cellular quiescence and homeostasis. These signals include soluble growth inhibitors that act via cell surface receptors and immobilized inhibitors that are embedded in the extracellular matrix and on the surface of nearby cells. The signals operate to either push the cell into G0 or into a postmitotic state (usually associated with the acquisition of specific differentiation–associated characteristics) and are thus intimately associated with cell cycle control mechanisms. Cells monitor their external environment during the progression through G1 and, on the basis of external stimuli, decide whether to proliferate, to become quiescent, or enter into a postmitotic state. At the basic level, most of the antiproliferative signals are funneled through the Rb protein and its close relatives. Disruption of Rb allows cell proliferation and renders the cell insensitive to

antiproliferative signals such as that provided by the well-characterized TGF-β.[29]

The development of the malignant phenotype, however, depends on more than the avoidance of cytostatic mechanisms. The cell also needs to avoid terminal differentiation and the postmitotic state. One example of how tumor cells manage this is provided by studies on the c-myc oncogene. This oncogene is a nuclear transcription factor that has growth-stimulating effects in association with another protein called Max. During normal development, the growth-inducing effect of myc-max complexes can be supplanted by alternative complexes of Max with a group of transcription factors known as Mad. Mad-Max complexes elicit signals for terminal differentiation. However, where the myc oncogene is amplified (as occurs in many tumor types), the balance of complexes is shifted in favor of myc-max, thereby impairing differentiation and promoting proliferation.

Evading Apoptosis or Programmed Cell Death

The growth of any tumor depends not only on the rate of cell division but also on the rate of cellular attrition (that is mainly provided by apoptotic mechanisms). Basic molecular and pathological studies of tumors have confirmed that an acquired resistance against apoptosis is a hallmark of all types of cancer.

Cancer cells, through a variety of strategies, can acquire resistance to apoptosis, and one of the most common is through loss of function of the tumor suppressor protein p53, discussed earlier.

Limitless Replicative Capacity

In the 1970s, the pioneering observations of Hayflick established that when normal human or animal cells are grown in culture, they demonstrate a finite replicative life span. That is, they are capable of a finite number of cell divisions, after which they undergo what has been termed replicative senescence and are incapable of any further cell division. The mechanism underlying the replicative clock that monitors this process has been the subject of intense research. This process has further evoked considerable interest as it is also one of the mechanisms that must be overcome to establish the immortal phenotype that is characteristic of the cancer cell.[40–42]

In mammalian cells, the DNA is organized into chromosomes within the nucleus, and these are capped by specialized DNA-protein structures known as telomeres. The major function of these structures is protection, but they are progressively eroded at each cell division because of the inability of DNA to completely replicate itself. The result is that there is progressive telomeric attrition as cell populations double. After an estimated 50 cell divisions,

cells enter an irreversible (and prolonged) state of cellular senescence (sometimes referred to as mortality stage 1 or M1). This period is characterized by arrest of proliferation without loss of biochemical function or viability. At the end of this period, cells exhibit altered morphology and chromosomal instability, a state often referred to as crisis (mortality stage 2, M2). See Figure 2-5. Thus, telomeric attrition is intimately involved with the aging of cells. Cancer cells must overcome replicative senescence and take on an immortal phenotype.

It has now been demonstrated in human tumors and, more recently, tumors of the dog that telomere maintenance is a feature of virtually all cancer types.[43–48] Tumor cells succeed in telomeric maintenance by the expression of the enzyme telomerase. From studies on cellular senescence, expression of the enzyme telomerase has emerged as a central unifying mechanism underlying the immortal phenotype of cancer cells and has thus become the most common marker of malignant cells. Telomerase is a ribonucleoprotein enzyme that maintains the protective structures at the ends of eukaryotic chromosomes, called telomeres (Figure 2-6). In humans, telomerase expression is repressed in most somatic tissues, and telomeres shorten with each progressive cell division. In contrast, telomerase activity is a common finding in many human malignancies resulting in stabilized telomere length. The telomerase complex consists of an RNA subunit that contains a domain complementary to the telomeric repeat sequence TTAGGG and a catalytic protein component. The catalytic protein component acts as a reverse transcriptase and can catalyze the addition of telomeric repeats onto the ends of chromosomes using the RNA subunit as a template. It is now well documented that the level of telomerase in malignant tissue compared to normal tissue is much higher and this differential is greater than that for classical enzymatic targets such as thymidylate synthase, dihydrofolate reductase, or topoisomerase II.

Telomerase biology is complex and the mechanisms by which telomerase becomes reactivated in tumor cells is the subject of intense research. However, this represents an exciting opportunity for further understanding the complex biology of cancer and also the identification of completely novel targets for therapy (See Chapter 14, Section D).

METASTASIS

Metastasis is defined as the dissemination of neoplastic cells to distant secondary (or higher order) sites, where they proliferate to form a macroscopic mass. Implicit in this process is the presence of a primary tumor. Metastases are not a direct extension of the primary tumor and are not dependent upon the route of spread (i.e., hematogenous versus lymphatic versus peritoneal
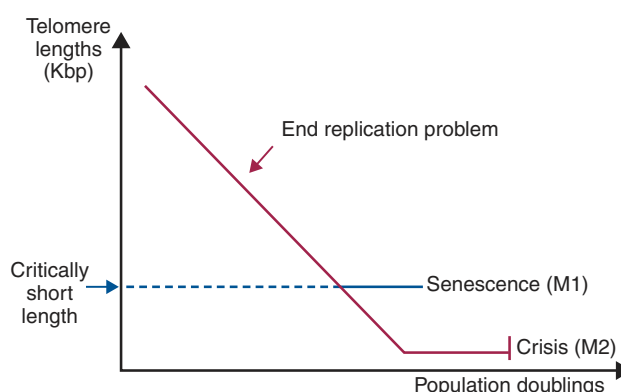
Figure 2-5

Telomeric attrition in cultured cells. In mammalian cells, the DNA is organized into chromosomes within the nucleus, and these are capped by specialized DNA-protein structures known as telomeres. The major function of these structures is protection, but they are progressively eroded at each cell division because of the inability of DNA to completely replicate itself. The result is that there is progressive telomeric attrition as cell populations double. After an estimated 50 cell divisions, cells enter an irreversible (and prolonged) state of cellular senescence (sometimes referred to as mortality stage 1, or M1). This period is characterized by arrest of proliferation without loss of biochemical function or viability. At the end of this period, cells exhibit altered morphology and chromosomal instability, a state often referred to as crisis (mortality stage 2, or M2).

Figure 2-6

The end replication problem. Telomeric attrition arises because of the inability of chromosomes to completely replicate their extreme 5′ ends (end replication problem).

seeding). The process of metastasis is believed to occur through the completion of a series of stepwise events (Figure 2-7). For this process to occur, a cancer cell must leave the site of the primary tumor, pass through the tumor basement membrane, and then through or between endothelial cells to enter the circulation
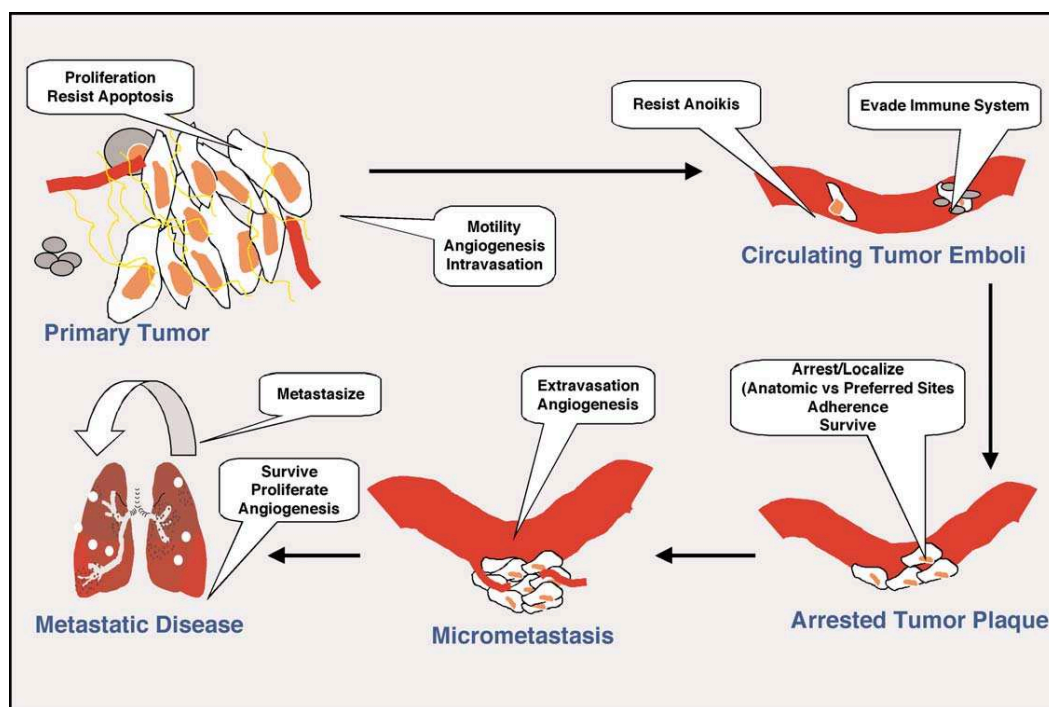
Figure 2-7

The metastatic cascade. The metastatic spread of cancer from primary tumor to distant sites requires the completion of multiple steps (processes). The primary tumor must be successful in proliferation and resistance of apoptosis. The **intravasation** of cells from the primary tumor into the vascular compartment involves coordinated degradation of local environment (e.g., protease-mediated MMPs), followed by directional **motility**, often along extracellular matrix filaments, toward vascular structures. Cells may enter the vasculature as single cells or as clumps of tumor cells that break free of the primary tumor. In the circulation, tumor cells (or emboli) must *evade damage and death* from physical forces of shear stress associated with rapid blood flow, immune-mediated destruction, and induction of cell death resulting from **anoikis** (death associated with loss of contact). Arrest at distant sites may be receptor mediated (i.e., laminin-V binding by integrins on tumor cells) or due to size-dependent trapping within small blood vessels. Following **extravasation** from the vascular space, cells must **survive** in the novel and often hostile microenvironment of the target organ. Successful survival requires modulation of this secondary micro-environment through the recruitment of stromal cells, the production of growth factors, and **angiogenesis**. An alternative mechanism of extravasation may include the **intravascular proliferation** of tumor cells. It is likely that the process of metastasis continues from established metastases to distinct metastases within the same organ or metastases within tertiary organs. As a result, all the steps of the metastatic cascade occur before and after a patient presents with a primary tumor or with established metastases.

(extravasation). While in the circulation, tumor cells must be able to *resist anoikis* (programmed cell death associated with loss of cellular contact), *evade immune recognition and physical stress,* and eventually *arrest at distant organs.* At that distant site, the cell must leave the circulation *and survive in the hostile microenviron-ment* of the foreign tissue. This distant site may be the eventual target organ for metastasis or may be a tempo-rary site. In either case, the cancer cell is thought to lie **dormant** for a protracted period of time before moving to its final location. Following dormancy, cells receive signals to **proliferate,** create new blood vessels (**angio-genesis**) or co-opt existing blood vessels and then successfully grow into a measurable metastatic lesion. It is likely that further progression is associated with the repetition of this process and the development of **metas-tases from metastases;** as such, the steps outlined here continue not only after the detection of the primary tumor but also after the detection of metastases. The basic tenants of this model of metastasis have been intact for since the 1960s; however, a greater under-standing of biological principles associated with each metastasis process is emerging. The opportunity provided by this emerging understanding is the develop-ment of novel strategies for the management of metas-tases in pet animals.

Metastasis-Associated Genes and Metastasis Suppressor Genes

Cancer cells are not unique in their ability to complete the individual steps required for metastasis. For example, leukocytes and neuronal cells have the ability to invade tissue planes and cross-vascular barriers. Several types of leukocytes demonstrate the phenotype of intermittent adherence to vascular endothelium and are able to resist anoikis. It is also true that stem cells, of various phases of differentiation, are able to perform many of these steps during development and in the adult.[49] What is unique about metastatic cells is that an individual metastatic cell must be able to perform all the steps required for successful metastasis. An extension of this argument is that the genetic changes that permit the metastatic process are not unique to a metastatic cancer cell; however, for the successful navigation through the metastatic cascade, a metastatic cancer cell must have an appropriate set of genetic changes.

Literally hundreds of genes and their resultant proteins have been suggested to contribute to the development of cancers and to their eventual ability to metastasize. It is possible for a single genetic change in cancer to contribute many of metastasis-associated processes or for several genes to work together toward a single metastasis-associated process. Different metastatic cancers may achieve the metastatic phenotype through distinct constellations of genetic events that in their respective sums complete the list of necessary metastasis-associated processes needed for successful metastasis. Two classes of genes have been broadly defined as contributing to the metastatic phenotype. These include metastasis promoting genes[50,51] and metastasis suppressors.[52,53] These genes have functions in normal development and physiology (i.e., cell migration, tissue invasion, and angiogenesis, discussed earlier) that are subverted by the cancer cell in the acquisition of the metastatic phenotype.

The use of high throughput and genome-wide investigations have uncovered many putative metastasis-associated genes in cancer. It should be noted that many metastasis-associated genes have functions that also contribute to tumor formation and progression. Several of these metastasis-associated genes have been validated in canine and feline cancers. For example, the metastasis-associated gene ezrin was identified using genomic approaches in murine and human studies of metastasis. Ezrin is a membrane-cytoskeleton linker protein that functionally and physically connects the actin cytoskeleton to the cell membrane. The expression of ezrin in the primary tumor of dogs with osteosarcoma, was shown to predict a more aggressive course of disease, defined by metastasis to the lung.[54]

Metastasis suppressor genes have been identified in several human cancers. The function of these genes has been reviewed elsewhere.[53] Suppressor genes are thought to regulate motility, invasion, angiogenesis, and other processes associated with metastasis. The loss of these genes is, by definition, not thought to be associated with the formation of a primary tumor. Examples of metastasis suppressor genes are listed in Table 2-2. Loss or reduced expression of metastasis suppressors has not been documented in canine or feline cancers at this time.

The following will provide descriptions of the critical metastasis-associated processes. Examples of genetic changes or resulting protein changes that contribute to each process will be highlighted with each section, with particular emphasis on those with demonstrated associations with malignancies in animals.

Intravasation

Following the successful growth of the primary tumor, intravasation of a cancer cell into the vascular or lymphatic circulation is the first step required during the metastatic cascade. The process of intravasation requires that a tumor cell be motile and able to digest its extracellular matrix.[55] Studies with intravital imaging (microscopy on live animals) have demonstrated that some cancers move along tracks formed by fibers within the extracellular matrix.[56] Interestingly, these studies have demonstrated that a minority of the cells in the primary tumor have this invasive phenotype. Efforts are underway to define the genetic features of this minority population.[57,58]

The protease family, specifically matrix metalloproteases (MMP), have been most commonly associated with the invasive phenotype. Expression of members of this enzyme family have been found in most human and several veterinary cancers, including osteosarcoma, mast cell tumors and lymphoma [59-63] The activity of MMPs in osteosarcoma and mast cell cancers has been correlated with grade and metastatic propensity.[61,62] Similar correlative studies have been undertaken in human patients.[64,65] The importance of MMP activity during the early steps in metastasis prompted the development of pharmacological inhibitors of MMPs. Anticancer activity of these agents in preclinical animal models and in sporadic human patients was evident; however, in randomized trials, the activity of MMP inhibitors was not supported.[64] Similarly disappointing studies in dogs with osteosarcoma and lymphoma have been completed (Barbara Kitchell, personal communication). The failure of these clinical trials may be explained at many levels and do not refute the importance of intravasation as a critical step for a metastatic cell.[66]

The process of intravasation concludes when a cancer cell successfully enters the vascular or lymphatic circulations. Tumor cells may enter the circulation through established blood vessels, small aterioles, venules, lymphatics, or through tumor-associated (or lined) blood vessels, in a process referred to as vasculogenic

TABLE 2-2   Metastasis Promoting Gene/Protein

Name	Function	METASTASIS-ASSOCIATED FUNCTIONS				
		Motility	Invasion	Arrest/Adhesion	Survival	Angiogenesis
Nm23	Signalling	X	X		X	
Breast cancer metastasis suppressor –1(BRMS1)	PIP Signalling		X	X	X	
Src-suppressed C kinase substrate (SSeCKs)	Signalling					
KiSS	Transmembrance receptor		X		X	
KAI1	Tetraspanin protein	X	X		X	
Melastatin	Cation channel	X	X			
CRSP3 transcriptional coactivator	Signalling		X		X	
Vitamin D3 up-regulated protein (VDUP)	Thioredoxin inhibitor		X		X	
Raf kinase inhibitor (RKIP)	Membrane phospholipid binding protein		X		X	X
Differentiation-related gene-1 (Drg-1)	Differentiation		X		X	
RhoGDI2	G-protein signalling	X	X			
MKK4	Signalling		X	X	X	
RECK	Membrane glycoprotein					
LGI1	Signalling		X			
E-cadherin	Adhesion molecule			X		

mimicry.[67] For larger vascular structures, the process of intravasation requires penetration of adventitial cells including pericytes, digestion of the vascular basement membrane, and penetration between or through endothelial cells.[68] Intravasation through tumor-associated vasculature may be easier and may only require transit from the extracellular space between endothelial cells into the circulation.

Survive in the Circulation (Resist Anoikis)

Frisch and Francis reported the induction of apoptosis after disruption of the interaction between epithelial cells and the extracellular matrix.[69] This phenomenon was termed *anoikis*, from the Greek for "homelessness." In normal tissues, anoikis is a major mechanism for maintaining tissue homeostasis and integrity. For the metastatic cancer cell, survival during dissemination requires resistance to anoikis. In normal tissues, anoikis is prevented by two systems: cell-matrix anchorage and cell-cell interactions.[70] Anchorage of cells to the extracellular matrix is mediated primarily by integrins.[71–73] These transmembrane receptors, consisting of an α chain and β chain, form active heterodimers upon ligation. Formation of active heterodimers triggers an intracellular cascade resulting in activation of effectors of growth

and survival.[73] Integrin family members have been identified in canine sarcomas and lymphomas.[74–78] Cell-cell anchorage in many epithelial tissues is mediated by cadherins, a family of calcium-binding glycoproteins. Intracellularly, cadherins form complexes with members of the catenin protein family that link them to the actin cytoskeleton, and survival promoting signal transduction cascades.[79] Loss of either cell-cell or cell-matrix interaction in normal cells triggers the activation of the caspase proteases, the hallmark of apoptotic cell death. Cancer cells may avoid anoikis by expression of proteins that directly inhibit anoikis. This overexpression subverts the need for ligand binding and results in the generation of survival factors,[80] independent of ligand. Proteins reported to be involved in resistance to anoikis include Trk B, focal adhesion kinase (FAK, the immediate effector of integrin signaling), galectin-3, and TGF-β.[81-84] Certainly more molecular mediators of anoikis resistance will be uncovered and will likely demonstrate tumor type specificity. These molecules may be valuable targets for novel antimetastasis therapy.

Evasion of the Immune System

At all stages of cancer progression, metastatic tumor cells must evade detection and destruction by the immune

system. The ability of the host immune system to recognize and destroy tumor cells, termed *immunosurveillance*, was first proposed by Paul Ehrlich in 1909.[85] Molecular support of the theory of immunosurveillance has come from studies of mice deficient in immunomodulatory and proinflammatory molecules such as interferon-γ (IFNγ), IL-12, and perforin. Mice deficient in these molecules are known to develop tumors more readily than wild-type mice.[86] Clinical evidence for immune-surveillance was first reported by Coley over 100 years ago. Through the administration of bacterial mixtures, Coley's toxin, Coley was able to induce fever and tumor regression in some patients with cancer. Anecdotal evidence in dogs with osteosarcoma further supports the potential value of immunotherapy. Withrow et al. have reported survival times in dogs who have developed bacterial infection at the site of a limb salvage surgery to be significantly longer than dogs who did not develop infection.[87] In immunocompetent hosts, it is believed that immune-surveillance removes a large number of cancer cells from the primary tumor, from the circulation and at distant metastatic sites. Cancer cells employ a wide variety of mechanisms to effect this evasion. The mechanisms for immune-surveillance and evasion from immune-surveillance by cancers are reviewed elsewhere and are summarized in Chapter 13.[88]

Modification of the immune system to treat cancer continues to be an attractive therapeutic strategy (See the review by Mocellin et al.[89]). Clinical trials based on this concept have been reported throughout the veterinary literature in dogs with melanoma, soft tissue sarcoma, hemangiosarcoma, osteosarcoma, and other tumors using a variety of immune based therapies.[90–93] The current direction of many immune-based therapies is toward the development of allogeneic or autologous tumor vaccines.[89,94] For the most part, the development of these vaccines is more advanced in veterinary clinical trials than in most human trials. Generation of vaccines directed against proteins overexpressed by specific tumors remains an attractive idea, since this therapy should be specific to the tumor and therefore associated with a low side effect profile.

Arrest in Target Tissues

The arrest of circulating tumor cells at distant sites is thought to occur by two distinct, but nonmutually exclusive, mechanisms. These include size dependent "trapping" of tumor cells within the lumen of small vessels (capillaries and venules) in the target organ or receptor-mediated interaction involving the tumor cell and the host vasculature.[95] Data supporting the "trapping" phenomenon come from work by Chambers et al.,[96–99] where single-cell imaging studies confirmed the fact that cancer cells primarily arrest at distant vascular beds as a result of size-dependent trapping of large tumor cells in

small blood vessels. This work was conducted in models of metastasis to the liver; however, similar results have been seen in other cancer types and other metastatic locations including the lung.[100] This trapping phenomenon suggests that the site for metastasis from a primary tumor is largely guided by the location of the first (small vessel) vascular bed encountered by a tumor cell. Alternatively, several groups have suggested the role of specific adhesion molecules as being necessary for initial tumor arrest. The work of Mushel et al. suggests that laminin V, a component of the endothelial basement membrane, is exposed between endothelial cells in small vessels, and is a significant target for integrin mediated adhesion.[101] Single-cell imaging studies in mice have demonstrated the binding of tumor cells to laminin V at these sites. It is likely that both of trapping and ligand receptor interactions play a role in the initial seeding of metastases. It is likely that the dominant mechanism of arrest is dependent on tumor type and target organ. The clinical significance of these mechanisms will be largely defined by novel cancer treatment strategies that are directed against receptor-mediated arrest of cancer cells.

Adhesion and Early Survival at Distant Sites

Once the cancer cell or cancer embolus has arrested at its distant sites, it may immediately move out of the circulation into the target organ or may stay within the circulation.[102] In either location, the cancer cell must survive in a new environment. This early metastatic survival is a significant hurdle for the cancer cell. Several studies in mice have shown the ability of cancer cells to arrest at multiple organs in the body. Within hours, the number of remaining cells is dramatically reduced; within days, the number of viable cells may be as low as 0.1% of the original number, even for the most aggressive cancer models.[54] The organ selectivity for cancers is to a large extent defined by the organs in which a cancer cell can survive following initial arrest. The "seed and soil" hypothesis first articulated by Paget; more recently, Fidler et al. suggested that the success of a cancer, or metastasis, is defined by interactions between the seed (tumor cell) and the soil (the tumor microenvironment). To a large extent, the seed and soil interactions that are responsible for early metastatic survival are provided by adhesion molecules. Arrest of tumor cells in target organs may or may not be receptor mediated (discussed earlier); however, adhesion requires receptor ligand interactions. Cell adhesion molecules (CAM) are thought to play a significant role in metastatic adhesion. Multiple CAMs have been identified and are named based on their cell specificity (e.g., N-CAM for neural and L-CAM for liver). CD44 is a specific adhesion molecule (H-CAM for homing-CAM), initially identified as

the receptor for hyaluronic acid on hematopoietic cells. Expression of splice variants of CD44 on tumor cells has been demonstrated to correlate with poor prognosis in a wide variety of human tumors from acute myelogenous leukemia and Hodgkin's disease to breast cancer and osteosarcoma.[103,104] Chemokines are a class of chemotactic cytokines that function in leukocyte trafficking and have been shown to contribute metastasis adhesion and other metastasis-associated processes. Chemokines may also enhance the survival of single metastatic cells that reach distant sites. The type and number of signaling adhesion molecules that contribute to metastatic success are distinct for each cancer and target organ.

Modulation of Novel Environment

Following survival at the distant site, the tumor cell must proliferate and modulate its new environment. In most cases it is believed that cancer cells extravasate from the circulation and then proliferate in the new organ. However, it is also possible for proliferation to occur within blood vessels, in a process referred to as intravascular metastases, and then expand out into the local tissue before further proliferation occurs.[105] In both situations, modulation of the new environment is necessary for appropriate growth and progression of the metastatic lesions. It is now recognized that part of this modulation is based on tumor-induced changes in the stroma.[106,107] These stromal changes may result in the production of growth factors or signals that are used by the tumor for further growth. Stromal cell-derived growth factors provide important signals for tumor cell proliferation and progression including angiogenesis.[106,108] The "induced" tumor-stroma may be a credible target for novel cancer therapeutics. Examples include inhibitors of the endothelin pathway.[109] The endothelin-I pathway is active in the interaction between tumor cells and osteoblasts at sites of bone metastases.[110,111] Endothelin (ET-I), produced by a number of tumor cells including cancers of breast and prostate, -I interacts with endothelin receptors (ET-A or ET-B) expressed on osteoblasts and on some tumor cells. The ET-I-induced activation signal then results in osteoblast release of growth factors such as bone morphogenic proteins (BMPs) that provide a tumor microenvironment conducive to successful metastasis survival and progression. Atracentan is a selective ET-A inhibitor in this class that has completed phase II trials, where its use improved time to progression in patients with advanced prostate cancers.[112] It is possible that the role of the ET-I pathway in metastasis will be generalizable to other cancers that metastasize to bone and primary tumors of bone such as osteosarcoma. In unpublished studies, we have shown that ET-A is expressed in canine osteosarcoma and that the metastatic outcome in these dogs is related to the intensity of ET-A expression in the primary tumor (Katherine Hansen, personal communication).

Angiogenesis

A key factor in tumor growth and metastasis is angiogenesis. Endothelial cells or endothelial progenitors are activated by tumor-derived growth factors and result in new capillaries at the tumor site.[113] In healthy tissue, endothelial cell proliferation is controlled by a balance between protein factors that activate endothelial cells and those that antagonize activation. Malignant tumors provide signals that result in endothelial cell survival, motility, invasion, differentiation, and organization. These steps are required to create a supportive vasculature for the tumor.[114,115] Reviews have summarized tumor-derived factors that support endothelial activation and function. In many ways, the processes required for endothelial activation and maturation parallel the features required for the success of a metastatic cancer cell itself. The creation of new blood vessels requires the tumors to recruit circulating endothelial cells to their site, presumably through the release of growth factors (e.g., vascular endothelial growth factor, or VEGF). Circulating endothelial cells must survive at their new site with the help of survival signals (e.g., thrombospondin-I, or TSP-I) and form vascular tubes that then reorganize to sustain blood flow. Many lines of evidence support the importance of angiogenesis in the biology of metastasis. The vascularity of a primary tumor (measured by microvessel density) has been correlated with metastatic behavior for most human and many veterinary tumors. The expression of angiogenesis-associated growth or survival factors and their receptors (i.e., VEGF-R) in serum and in tumors, respectively, has also been correlated with outcome, and more recently functional imaging studies using MRI and other means have provided correlates of vascularity with poor outcome.[116] The strength of this biological argument has supported the development of a number of novel therapeutic agents with antiangiogenic activities (See Chapter 14, Section E).[117–119] These agents have moved through discovery and development and are now appearing as approved drugs for cancer.

Metastasis from Metastases

For most solid tumors, the appearance of a single metastatic nodule is followed shortly by the development of additional metastases within the same target organ and tertiary sites. It is unlikely that these metastatic sites all emerge from distinct and unique clones from within the primary tumor and nearly simultaneously progress through the metastatic cascade to yield synchronous metastases at the distant site. It is reasonable and perhaps more likely that metastases also develop from other metastatic sites. In this hypothesis, a small number of successful clones colonize distant sites. As these clones become successful metastases, the process of metastasis continues, resulting in metastasis from metastases. Although data supporting

this hypothesis are limited, the implication for the treatment and management of cancer patients is great. If true, the process of metastasis from metastases would suggest that all steps in the metastatic cascade occur continuously, both before and after detection of metastases in patients. As such, all the steps in the metastatic cascade may be targets for future therapeutic intervention.

Controversies in the Field

The foregoing discussion represents a consensus on the steps associated with the model of the metastatic cascade; however, areas of active debate and investigation in the field continue to evolve. The following section highlights questions that remain in the field.

Does the Metastatic Propensity for Tumors Emerge Early or Late in the Biology of Cancer?

The development of the metastatic phenotype has been traditionally believed to be a process that happens late in carcinogenesis. In this model, referred to herein as the "progression model" (Figure 2-8), the genetic changes responsible for primary tumor development are in most cases distinct and precede those steps that contribute to the metastatic phenotype.[120–122] The progression model argues that the metastatic phenotype is acquired within a small fraction of cells within the heterogeneous primary tumor. Support for the progression model came from work by Fidler and others, who demonstrated the ability to select for rodent cancers with greater metastatic potential through the serial passage of metastatic tumor nodules back to naïve mice.[121] This selection phenomenon suggested that a minority of tumor cell clones within a primary tumor were endowed with the metastatic phenotype and that this small proportion were enriched in the metastases compared to the primary tumor. Application of the progression model would suggest that a period of time exists between primary tumor development and the acquisition of the full metastatic phenotype. This model was thought to be the basis of the improved outcome associated with early detection of cancers and the belief that effective and definitive therapy was most likely if a diagnosis was made early. Recent work has provided data to support an alternative model.[50] Using expression microarray, the presence of a metastatic signature was found within primary tumors. Detection of a profile by expression array suggest that the metastatic signature must be present in more than
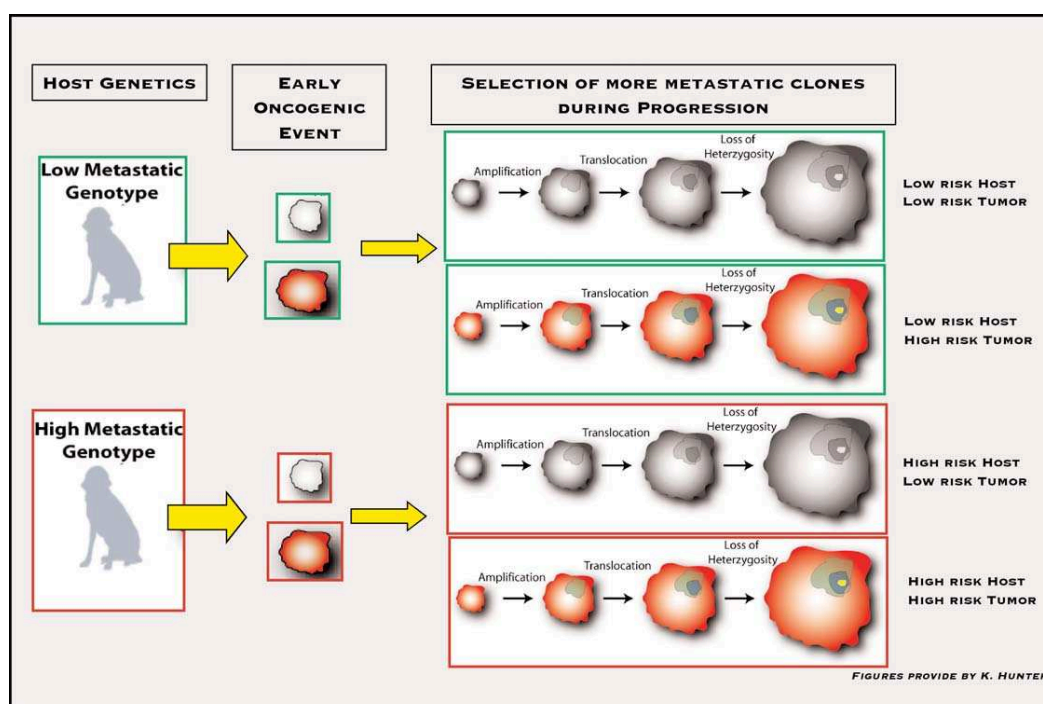
Figure 2-8

Emergence of the metastatic phenotype. The emergence of the metastatic phenotype is influenced by (1) host genetics, (2) early oncogenic events that are related to initial carcinogenesis, and the (3) selective acquisition of metastatic cells within the primary tumor. The relative contribution made by each to the total metastatic biology of cells is not known and is likely to be distinct for each cancer type and patient.

a minority of the cells and the possibility that this pheno-type emerges earlier in the carcinogenesis of the cancer than predicted by the progression model. This alternate model, "the early oncogenic model" (See Figure 2-8) for metastasis, suggests that the genetic events that contribute to carcinogenesis are the same or emerge at the same time as the events that contribute to the metastatic phenotype. Application of the early oncogenic model suggests that the biology of a cancer is defined early and as such may not be something that can be reduced through the early identification of a cancer. This is not to say that early detection of a cancer is not helpful for a patient, but rather that bad cancers may be "born" bad. This model may explain the phenomenon of the unknown primary tumor, wherein metastatic disease is detected without an apparent primary tumor. To add complexity to this question, there is increasing evidence that host (genomic) differences can influence metastatic behavior of cancers, without necessarily influencing primary tumor growth or cancer predilection.[123-124] Using a genetically engineered mouse model of mammary cancer, Hunter was able to identify host genes that influence metastatic behavior of tumors. This work has shown that a significant number of differentially regu-lated genes that define the metastatic signature of tumors, discussed earlier, are in fact modulated by host genetic polymorphisms rather than tumor specific genomic events. These findings have several important implications. First, they suggest that individuals might be predisposed to aggressive metastatic progression before the development of a tumor. In addition, it suggests that there may be families in the population that are at high risk, not necessarily for tumor develop-ment but for an aggressive metastatic course once a tumor develops. Most important, if a significant part of a patient's metastatic risk is encoded within the patient's constitutional genome rather than within the mutated tumor genome, it may be feasible to identify those individuals at high risk for metastatic disease at the time of diagnosis of the primary tumor, or poten-tially before. These findings may be particularly relevant in the field of veterinary oncology, where breed-associated genomic differences may explain the more aggressive course of disease seen in some dogs compared to others. Taken together the risk for metastatic progression is in part defined by the genetics of the patient; genetic changes that develop early in the process of tumor devel-opment and the subsequent and incremental emergence of aggressive metastatic cells (see Figure 2-8).

Where Is the Inefficiency in Metastatic Inefficiency?

As devastating as the metastatic process is, it is equally inefficient. Estimates suggest that less than 1% of highly metastatic osteosarcoma cells that successfully enter the circulation are able to survive in the lung.[98,125] The true metastatic inefficiency of human and veterinary cancers is likely to be much lower. What is not clear is where in the complicated metastatic cascade does this inefficiency occur? It does not seem that entry into the circulation is the major barrier for successful metastases. Studies have identified high numbers of circulating tumor cells in cancer patients who are free of metastatic disease. The clinical importance of high circulating tumor cell numbers is not clear;[126] however, it appears that for some cancers, high circulating numbers does not neces-sarily correlate with risk for metastasis. Bulter and Gullino estimated that between $1–4 \times 10^6$ cells/gram of tumor enter the systemic circulation each day in human cancers.[127] These data suggest that although intravasa-tion is necessary for metastasis, it is not sufficient nor is it rate limiting. Although arrest at target organs may be a limitation for some cancers, the successful early survival of cancer cells at distant sites appears to be a major hurdle for successful metastases and therefore is a significant contributor to metastatic inefficiency. Individual metastatic cancer cells or metastatic tumor emboli do not have the paracrine growth factor and survival signal environment of the primary tumor and as such are particularly sensitive to cell death. If early and effective communication with the new environ-ment is established, cells can begin to proliferate and modulate the microenvironment of the secondary site, including the recruitment of vasculature needed to sustain and promote metastatic tumor growth. It is possible that the most inefficient or rate limiting steps in the metastatic cascade may be a valuable process to target for novel therapy since the natural barriers for successful cancer metastasis are already high.[97,128] The corollary of this is that targeting efficient steps in the metastatic cascade may not lead to significant impacts on treatment outcome.

What Is Dormancy and Where Do Dormant Cells Reside?

Despite effective control of the primary tumor and aggres-sive multiagent and multimodality adjuvant therapy, the risk for metastases to distant sites remains high for several cancer histologies. Since most patients are free of gross metastases at the time of diagnosis, the develop-ment of metastases is presumed to emerge from micro-scopic metastases. The location, size, angiogenic state, and proliferative/apoptotic state of these microscopic metastases is largely unknown. Histological studies of organs at risk for metastases in human cancer patients have identified small clusters of cancer cells that are poorly vascularized.[129,130] These findings have led to the common understanding of microscopic metastases as small clusters of tumor cells that reside within the organ of eventual metastasis. These microscopic metastases may exist in a preangiogenic state, either quiescent (outside the cell cycle) or in a balanced state of proliferation and

apoptosis. The signals that induce these presumed small populations of cells to emerge into gross metastases are not known. Data from murine models of metastasis, however, suggest an alternate model. These studies suggest that microscopic metastases consist of single "dormant" microscopic cells that may reside within a transient premetastatic reservoir (i.e., bone marrow). Subsequently these dormant cells "break" dormancy and transit from the reservoir site to the target organ. In support of this alternative model of dormancy, single disseminated tumor cells have been detected in the bone marrow in human cancers that are not known to result in clinical bone marrow infiltration. Insights gleaned from stem cell biology provide further support for the hypothesis that dormant metastatic cells are capable of residence in transient sites like the bone marrow, where they may rest in a dormant state before being recruited into the metastatic cascade.

The Role of Genome Instability

In this discussion we have placed decades of fundamental cancer research into six neatly defined boxes, each representing the capabilities that a normal cell needs to acquire to show the hallmarks of malignancy. Most of these capabilities require changes in the genome through mutation, amplification, or chromosomal translocation. However, the process of mutation is, in fact, inefficient because of the complex and fastidious maintenance mechanisms of the normal cell that monitor DNA damage and regulate repair enzymes. It is therefore actually difficult to explain why cancers arise in animals at all, as the acquisition of all traits would seem an impossible task. It may be argued that genomes must attain increased mutability in order to develop into a cancer cell.[131,132] Alternatively, it may be that the caretaker mechanisms are affected first and explain increased mutability. In particular, loss of p53 function leads to failure of the cell to arrest or apoptose in response to DNA damage. We have not included genomic instability in the categories described earlier as we consider that it represents the means that enable evolving premalignant cells to reach these six biological end points.

The Pathway to Cancer

It is important to stress that the pathways for cells becoming malignant are highly variable. Mutations in certain oncogenes can occur early in the progression of some tumors and late in others. As a consequence, the acquisition of the essential cancer characteristics may appear at different times in the progression of different cancers. Furthermore, in certain tumors, a specific genetic event may, on its own, contribute only partially to the acquisition of a single capability, while in others it may contribute to the simultaneous acquisition of multiple capabilities. However, irrespective of the path taken, the hallmark capabilities of cancer will remain common for multiple cancer types and will help clarify mechanisms, prognosis, and the development of new treatments.

Cancer Stem Cells

It was first extensively documented for leukemia and multiple myeloma that only a small subset of cancer cells is capable of extensive proliferation. When mouse myeloma cells were obtained from mouse ascites, separated from normal hematopoietic cells, and put in clonal *in vitro* colony-forming assays, only 1 in 100 to 1 in 10,000 cancer cells were able to form colonies.[133] Even when leukemic cells were transplanted *in vivo*, only 1% to 4% of cells could form colonies in the spleen.[134] Because the differences in clonogenicity among the leukemia cells mirrored the differences in clonogenicity among normal haematopoietic cells, the clonogenic leukemic cells were described as leukemic stem cells. It has also been shown for solid cancers that cancer cells are phenotypically heterogeneous and that only a small proportion are clonogenic in culture and *in vivo*.[135,136] For example, only 1 in 1000 to 1 in 5000 lung cancer, ovarian cancer, or neuroblastoma cells were found to form colonies in soft agar.[137] Just as in the context of leukemic stem cells, these observations led to the hypothesis that only a few cancer cells are actually tumorigenic and that these tumorigenic cells could be considered as cancer stem cells.[138]

If the growth of solid cancers is driven by cancer stem cells, it would have profound implications for cancer therapy. At present, all of the phenotypically diverse cancer cells are treated as though they have unlimited proliferative potential and can acquire the ability to metastasize. For many years, however, it has been recognized that small numbers of disseminated cancer cells can be detected at sites distant from primary tumors in patients that never manifest metastatic disease.[139] One possibility is that immune surveillance is highly effective at killing disseminated cancer cells before they can form a detectable tumor. Another possibility is that most cancer cells lack the ability to form a new tumor such that only the dissemination of rare cancer stem cells can lead to metastatic disease. If so, the goal of therapy must be to identify and kill this cancer stem cell population. If solid cancer stem cells can be identified prospectively and isolated, then we should be able to identify more efficiently new diagnostic markers and therapeutic targets expressed by the stem cells. The cancer stem cell theory might explain the failure to develop therapies that are consistently able to eradicate solid tumors. Although currently available drugs can shrink metastatic tumors, these effects are usually transient and often do not appreciably extend the life of patients.[140] One reason for these treatment failures is the acquisition of

drug resistance by the cancer cells as they evolve; another possibility is that existing therapies fail to kill cancer stem cells effectively.

Existing cancer therapies are targeted largely against the bulk population of tumor cells because they were identified by their ability to shrink tumors. Because most cells in a cancer have limited proliferative potential, an ability to shrink a tumor mainly reflects an ability to kill these cells. Normal stem cells from various tissues tend to be more resistant to chemotherapeutics than mature cell types from the same tissues.[139] The reasons for this are not clear, but may relate to high levels of expression of antiapoptotic proteins or ABC transporters such as the multidrug resistance gene.[139] If the same were true of cancer stem cells, then one would predict that these cells would also be more resistant to chemotherapeutics than tumor cells with limited proliferative potential. Therapies that cause complete clinical regression of tumors might spare enough cancer stem cells to allow regrowth of the tumors. Therapies that are more specifically directed against cancer stem cells might result in more durable responses and even cures of metastatic tumors. The concept of cancer stem cells is an exciting one, and prospective studies should be employed in veterinary cancer patients to identify these populations.

Genetic Predisposition to Cancer

Humans have a number of inherited syndromes that give rise to familial cancer syndromes (See Chapter 1A). The best characterized are Li-Fraumeni syndrome (inheritance of an abnormal copy of the p53 allele) and retinoblastoma (inheritance of an abnormal copy of the Rb allele). In both of these syndromes, the defect is in a tumor suppressor gene and therefore requires that both alleles are lost for abnormal function. However, affected individuals are more likely to develop cancers at a younger age. Other inherited cancers include Wilm's tumor (WT1), familial adenomatous polyposis (FAP), and breast cancer (BRCA 1 and BRCA 2). It is well recognized that certain breeds of dogs have a predisposition to certain cancers.[140] A putative hereditary cancer gene has been identified in German shepherd dogs that develop renal cystadenocarcinomas. Interestingly, this disease and the associated gene have strong similarities to a human cancer syndrome (Britte-Hogge-Dubay syndrome). A germ line mutation in p53 has been identified in a bull mastiff with lymphoma. This breed has a particular predisposition to lymphoid neoplasia.[141]

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3

The Pathology of Neoplasia

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Veterinary pathologists play a critical role in the management of neoplasia of pet animals by providing accurate diagnostic information to clinicians so that a prognosis can be determined and adequate treatment provided. The clinician needs to be knowledgeable about the pathology of neoplasia to understand neoplastic conditions and the limitations of histopathologic assessment in the diagnosis of neoplasia. The pathologist and the clinician must work together to determine optimal treatment for the patient, because the diagnosis and treatment of neoplasia in veterinary medicine have become increasingly complex. Simply determining whether the tumor is benign or malignant is no longer sufficient. The tumor type needs to be identified as accurately as possible, and tumor subtypes should be identified if prognostically significant. Grading of tumors has become increasingly important, because the behavior of some tumors has been shown to be predicted by grade. The assessment of margins for completeness of surgical removal is equally important. In some cases, the histologic assessment of tumors treated preoperatively is important for predicting treatment outcome. Special procedures such as immunohistochemistry, electron microscopy, flow cytometry, or polymerase chain reaction (PCR) may be advantageous in some cases to correctly identify the tumor type or subtype or to predict clinical behavior. Classification of neoplasia in veterinary medicine is not as advanced as in human medicine, but rapid strides in this direction are continuously being made. As more is learned about the diagnosis and treatment of neoplasia in veterinary medicine, new predictive classifications and grading schemes will continue to develop.

SAMPLE HANDLING

The biopsy sample should be visually inspected by the clinician to ensure that the appropriate tissue was obtained. If the biopsy sample was a needle core or incisional specimen, it should be of sufficient size and consistency so that it does not fall apart in formalin or become lost in processing. Samples less than 1 mm^3 are usually inadequate, although a needle core sample 1 mm wide and at least 5 mm long can be sufficient. If the biopsy samples are needle core samples, several cores should be obtained, if possible.

Very small samples can be lost during shipping or in processing, because samples shrink during fixation and processing. Given these limitations, some techniques can be used to maximize sample adequacy. Samples less than 3 mm^3 can be placed on paper (surgical glove paper is appropriate) before fixation. These samples will be tacky and adhere to the paper. Very small or pale samples can be circled with pencil to draw attention to them at the histology laboratory. The paper can then be folded around the sample and the whole package placed in formalin for fixation and shipping. Alternatively, commercially available screened tissue cassettes can be used to house the sample during fixation and shipment. The sample is placed in the screened cassette at the time of surgery and then placed directly into formalin. These techniques reduce the chance that small samples will be lost in larger formalin containers. Small samples can also be dyed with india ink or some other commercially available dye to assist identification of the sample. Samples containing excessive blood, mucus, or necrotic material may not be diagnostic, and the biopsy procedure may need to be repeated. If the specimen is an excisional sample, the entire sample should be submitted, if feasible, and the margins of concern identified with suture or ink. For very large samples, such as large splenic tumors, representative sections from both the periphery and the center should be included, along with samples from all different-colored or different-textured areas. At least three to five sections of large lesions should be submitted, in case some portions of the tumor have excessive distortion, necrosis, or inflammation. When representative samples are taken, the tumor and normal tissue interface should be included so that tumor invasiveness into normal tissue can be assessed. Tissue samples should

be handled gently, because compression during biopsy sampling and excessive use of electrocautery, cryosurgery, or laser surgery can cause destructive specimen artifact.[1]

The sample must be preserved in fixative. The most widely used fixative is 10% neutral buffered formalin, which is readily available and frequently supplied in individual specimen containers by most laboratories. In excessively cold conditions, samples can freeze during shipment and cause significant destructive tissue artifact. Adding 20% ethylene glycol or ethanol to the formalin can prevent freezing and maintain tissue integrity. Before immersion in the fixative, larger samples may need to be sliced, facilitating adequate fixation; however, the deep edge should be left intact, rather than slicing all the way through the sample, so that orientation and margin assessment are not compromised. Slices should be at least 1 cm thick, because curling and distortion during fixation can occur with thinner slices. The volume of tissue to fixative should approximate 1:10. When this ratio is not feasible because of large tumor size, multiple representative sections can be obtained. It is advisable to save the remnants of the sample, if possible, in case the first sections are not adequate for a diagnosis. When large samples are to be mailed, smaller volumes of fixative may be used if the specimen has been in the recommended volume for at least 12 hours.[2]

Sample containers should be properly labeled (on the container, not just the lid) before submission. Paperwork can be inadvertently separated from the sample container during transportation or unpacking at the laboratory; proper labeling prevents subsequent confusion. Equally important, an adequate history, including the signalment, pertinent clinical findings, radiographic findings, and treatment history, should be provided to the pathologist. A drawing of the sample indicating the position of the tissue in the animal is helpful in some situations, especially when margin determination is needed. If margins or areas of special clinical interest are marked (labeled) on the sample, a corresponding clear description of these labels should be present on the submission form. A proper history is crucial for accurate diagnosis; without it, the pathologist can be severely handicapped, and the end result may be an inaccurate diagnosis, culminating in an inaccurate prognosis and improper treatment.[1,3]

At the laboratory, the specimens are catalogued and assigned an identification number, visually examined, trimmed to fit into processing cassettes, processed into paraffin blocks, sectioned, and stained. Most of these procedures are done by trained technicians and automated laboratory equipment. In most laboratories, tissues are trimmed into cassettes on the day of arrival and processed overnight, and completed slides are ready for examination by the pathologist 24 hours after receipt. Hard tissue, such as bone, must be decalcified before sectioning and takes longer to process. Larger incompletely fixed samples,

extremely bloody samples (e.g., spleen), or samples with abundant fatty tissue (e.g., mammary gland) may require additional time for fixation. This extra time is vital to ensure that fatty tissue at the margin of the surgically excised sample remains intact during trimming, processing and sectioning. Otherwise, in the processes of trimming in or sectioning on the microtome, margins could become distorted and orientation may be lost. The pathologist then may have to re-examine the gross specimen. Most laboratories hold remnant wet tissues in formalin for 7 to 60 days in case further examination or sectioning is needed. Most laboratories file paraffin blocks and glass slides indefinitely, permitting review of previously submitted tissue on a particular patient or retrospective studies on a series of cases.

Although infrequently done, frozen sections can be made during operative procedures to provide the surgeon with a more rapid diagnosis. Samples are quick frozen, sectioned on a cryostat, fixed, stained, and examined within 20 to 35 minutes. Because this technique is performed during surgery to assist intraoperative decision making, a diagnostic laboratory and facility are required on site. However, these tissue sections are inferior to those processed routinely into paraffin, and as a result, diagnostic accuracy is not as high. Furthermore, few veterinary institutions provide this service, and not all veterinary pathologists are experienced in interpretation of frozen sections. This procedure may be helpful for establishing the identity of the tissue, the adequacy of surgical margins, or the adequacy of the tissue for more routine processing. Sometimes a provisional diagnosis or at least a distinction between benign and malignant processes can be made. The frozen-section diagnosis is always confirmed by routine histopathologic assessment of paraffin-embedded sections, often using the same tissue sample.[2]

Molecular diagnostic techniques (see Chapter 8) are becoming more routine in veterinary medicine. PCR and immunohistochemistry (IHC) procedures have been developed and characterized for use on formalin-fixed, paraffin-embedded samples. Once processed, such samples are often viable indefinitely for these tests, although the time before fixation, the time in fixation, and the storage time can impair test sensitivity. These factors need to be determined for individual tests, and their effect must be considered in the interpretation of results. IHC techniques on formalin-fixed tissues require unstained, routine sections on slides appropriate for IHC. Exposure to sunlight or extremes of temperature should be avoided. PCR techniques require thick sections (10 to 20 μm) to ensure adequate amounts of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). These sections typically are allowed to roll up during microtome sectioning and can be sent to the testing laboratory at room temperature in an airtight container. Requirements particular to individual testing

laboratories should be determined before sample collection and submission to prevent frustration for the owner, submitter, and testing laboratory.

TERMINOLOGY

Numerous terms are used to describe the features of a tissue sample. A clear understanding of this terminology is imperative if the clinician is to understand the implications of the histopathologic findings and be able to discuss details of a tumor with the pathologist. The clinician's responsibility extends beyond collecting the tumor sample and treating the patient; the clinician must also interpret the histopathology report, identifying details that help determine a tailored treatment protocol for the individual patient.

A *tumor* is any tissue mass or swelling; it may or may not be neoplastic. *Neoplasia* is the abnormal growth of a tissue into a mass that is not responsive to normal control mechanisms; it may be benign or malignant. The growth of this mass is not affected when the inciting stimulus is removed. The term *cancer* refers to a malignant neoplasm.[4] All neoplasms arise in normal tissue and therefore are composed of parenchymal and stromal cells; some can also incite secondary inflammation. A neoplasm's state of differentiation can be assessed with histopathology. It is based on the appearance of the neoplastic cells, their organization, and their association with the supporting stroma. Normal, reversible processes of *hyperplasia* (a nonneoplastic increase in the number of cells) and *atrophy* (a decrease in the number of cells) also involve parenchymal cells and stroma, and the retention of near normal architecture in such structures can make their differentiation from well-differentiated neoplasms difficult. At times, only removal of the inciting stimulus and time can distinguish between hyperplasia and well-differentiated cancer.

Cancer has also been defined as a proliferation of a clonal population of cells that is no longer responsive to tissue homeostatic mechanisms. Molecular techniques to determine a clonal expansion of cells by their similar DNA sequence structure can help identify these conditions or detect occult cancer before tissue distortion can be seen at a microscopic level. Molecular assays of clonality have been developed for some tumors, more specifically for use in canine and feline lymphoma.[5]

Metaplasia is the abnormal transformation of a differentiated tissue of one kind into a differentiated tissue of another kind; it is not a neoplastic condition. Metaplasia should reverse (or not progress) with cessation of the chronic inciting stimulus. An example is squamous metaplasia in the prostate gland, where normally columnar epithelium becomes squamous under the influence of estrogen. Metaplastic cells can become targets for carcinogenesis if continued carcinogenic promotional events occur (e.g., bronchial squamous metaplasia in

smokers). In such cases, metaplasia often progresses and acquires dysplastic changes. *Dysplasia* is abnormal tissue development; it can be a feature of neoplasia but is not necessarily a neoplastic condition. In some cases dysplasia, such as epithelial dysplasia in the oral cavity, can be a preneoplastic condition. *Anaplasia* is a loss of differentiation or atypical differentiation; it is a feature of many but not all malignancies.

Terms associated with cellular or growth features are frequently encountered in descriptions of neoplasia. *Pleomorphism* is the occurrence of multiple forms, shapes, and sizes of cells (cellular pleomorphism) and nuclei (nuclear pleomorphism). *Anisocytosis* and *anisokaryosis* are greater than normal variations in cell size and nucleus size, respectively. Round or polygonal cell shapes usually are associated with epithelial or hematologic tumors, whereas spindloid cell shapes usually are associated with mesenchymal tumors. A *scirrhous*, or *desmoplastic*, response is an abundant fibroblastic proliferation with collagen formation that occurs in some malignant invasive cancers. *In situ* refers to a malignancy, usually limited to lesions of epithelial origin, that has not yet become invasive beyond the natural confines of its basement membrane.[1,3,4]

For each type of tumor, specific terminology is used to denote the tumor's origin and whether the tumor is benign or malignant (Table 3-1). In basic terms, although benign tumors can cause morbidity through tissue distortion, they typically do not result in high mortality. In contrast, malignant tumors (cancer) are more destructive of tissues and often can lead to death if the patient goes untreated. However, exceptions to these rules exist. Because tumors can develop from any normal tissue type, there are a considerable number of tumor types. As more is understood about certain tumors, names and subclassifications may change, creating some confusion. A benign tumor of epithelial origin is called an *adenoma*, a *papilloma*, or an *epithelioma*. Benign tumors of mesenchymal origin are designated by the suffix *-oma* after the tissue type (e.g., fibroma, osteoma). A malignant tumor of epithelial origin is called a *carcinoma*, or an *adenocarcinoma* if the tumor forms glands and ducts; a malignant tumor of mesenchymal origin is called a *sarcoma*. In some cases the *-oma* suffix is used when the tumor is malignant (e.g., malignant melanoma and lymphoma). *Leukemia*, a malignant neoplasia of white blood cells in hematopoietic tissues and usually in the blood, has no benign counterpart, although a *leukemoid reaction* is a nonmalignant condition that mimics leukemia.[1,3,6]

HISTOLOGIC FEATURES OF NEOPLASIA

Despite recent advances in a number of areas of pathology, including molecular techniques, evaluation of tissue by light microscopy remains the standard for

TABLE 3-1   Nomenclature of Common Tumor Types in Veterinary Medicine

Tissue or Cell of Origin	Benign	Malignant
Epithelial		
Squamous	Squamous papilloma	Squamous cell carcinoma
Transitional	Transitional papilloma	Transitional cell carcinoma
Glandular	Adenoma, cystadenoma	Adenocarcinoma
Nonglandular	Adenoma	Carcinoma
Mesenchymal		
Fibrous tissue	Fibroma	Fibrosarcoma
Fat	Lipoma, "infiltrative lipoma"	Liposarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteosarcoma, multilobular osteochondrosarcoma
Muscle (smooth)	Leiomyoma	Leiomyosarcoma
Muscle (skeletal)	Rhabdomyoma	Rhabdomyosarcoma
Endothelial cells	Hemangioma	Hemangiosarcoma
Synovium	—	Synovial cell sarcoma
Mesothelium	—	Mesothelioma
Melanocytes	Benign melanoma, melanocytoma	Malignant melanoma, melanosarcoma
Peripheral nerve	Schwannoma, neurofibroma	Malignant schwannoma, neurofibrosarcoma, peripheral nerve sheath tumor
Uncertain origin[a]	—	Malignant fibrous histiocytoma, hemangiopericytoma
Hematopoietic and lymphoreticular		
Lymphocytes	—	Lymphoma: lymphoblastic or lymphocytic leukemia
Plasma cells	Cutaneous plasmacytoma	Multiple myeloma
Granulocytes	—	Myeloid leukemia
Red blood cells	—	Erythroid leukemia
Macrophages	Histiocytoma	Malignant histiocytosis
Mast cells	—	Mast cell tumor[b]
Thymus	Thymoma, encapsulated	Invasive thymoma
Brain[c]		
Glial cells	Astrocytoma, oligodendroglioma	Astrocytoma, glioblastoma multiforme, oligodendroglioma
Meninges	Meningioma	Malignant meningioma
Gonadal		
Germ cells[c]	Seminoma, dysgerminoma	Seminoma, dysgerminoma
Supportive cells[c]	Sertoli cell tumor, granulosa cell tumor	Sertoli cell tumor, granulosa cell tumor
Interstitial cells	Interstitial (Leydig) cell tumor, thecoma, luteoma	—

[a]Pathologists disagree about the origin of these tumors, some feeling they are a class of peripheral nerve sheath tumors.

[b]Theoretically all mast cell tumors are potentially malignant, but grade 1 mast cell tumors are clinically benign.

[c]Unfortunately, the terminology of these tumors does not distinguish between benign and malignant forms.

tumor diagnosis.[2] Neoplasia has certain histologic features that distinguish it from hyperplasia or inflammation, and some features distinguish benign from malignant neoplasia. However, these features sometimes can be difficult to observe. Definitive diagnosis of malignant versus benign versus inflammation or hyperplasia may

not always be possible. In these cases, a repeat biopsy, done either immediately or after a period of clinical observation, may facilitate a definitive diagnosis.

When inflammation is present, the cellular features of reactive fibroblasts and reactive endothelial cells can be misleading.[1,7] However, in reactive tissue with

inflammation, the fibroblasts and endothelial cells usually are oriented perpendicular to one another (reactive granulation tissue), and a substantial amount of inflammation relative to reactive tissue usually is present. When granulomatous inflammation occurs, large reactive and epithelioid macrophages can be mistaken for tumor cells, but the pattern of tissue involvement and the presence of other inflammatory cells help to rule out neoplasia. In some tumors, especially those with surface ulceration or extensive necrosis, some synovial cell sarcomas, and some soft tissue sarcomas, extensive secondary inflammation can obscure the visualization of neoplastic cells.

Benign tumors may be most difficult to distinguish from hyperplasia (Table 3-2) because both have a proliferation of well-differentiated cells that are easy to identify. Distortion or loss of normal tissue architecture occurs in benign neoplasia, and the tumor usually grows in an expansive manner, causing compression rather than invasion of adjacent tissue. These tumors often are defined by a fibrous tumor capsule. Hyperplasia tends to retain normal tissue orientation and does not compress adjacent tissue. It often lacks a fibrous capsule. In general, if allowed to grow, benign neoplasia attains a larger size than a hyperplastic lesion. In some cases the distinction between a benign tumor and hyperplasia is not clinically important; examples include thyroid gland adenoma versus adenomatous hyperplasia of the thyroid gland in cats and sebaceous gland adenoma versus sebaceous gland hyperplasia.

Features that distinguish malignant from benign neoplasia include a more dramatic loss of tissue organization, increased anisocytosis and anisokaryosis, increased nuclear and cellular pleomorphism, an increased and variable nucleus to cytoplasm ratio, abnormal nuclear chromatin, increased and abnormal mitotic figures, abnormal large and/or multiple nucleoli, increased necrosis, the amount and character of the supporting stroma, and the extent of invasiveness (see Table 3-2). With invasion, individual cells or groups of tumor cells infiltrate extensively into surrounding tissue, may invade vascular or lymphatic spaces, and may evoke a scirrhous (desmoplastic) response characterized by an excessive fibrous reaction. Another feature of malignancy is destruction of normal surrounding tissue or obliteration of normal tissue architecture. Evidence of lymph node or distant metastasis obviously distinguishes malignant from benign tumors.[1,3,8] However, in certain tumors, the histologic features do not correlate with behavior. Examples of this include canine histiocytoma and benign plasmacytoma. Both have histologic features of malignancy but are clinically benign. Histologically low-grade yet biologically high-grade fibrosarcomas of the canine head have histologic features of a benign condition but are clinically malignant.[9] Similarly, bronchial carcinomas in cats retain organized epithelial structures composed of well-differentiated, ciliated, pseudostratified columnar epithelium even at distant metastatic sites, including the digit, eye, heart, and kidneys.[10] In these cases, a knowledge of the clinical history and tumor behavior is necessary to distinguish benign from malignant neoplasia.

Generally, a pathologist makes the diagnosis of tumor versus reactive tissue and sometimes tumor type at relatively low magnification after evaluating the overall tissue pattern and behavior with respect to adjacent normal tissue. Higher magnification is then used to confirm the low magnification impression, to classify the tumor type if not already done, and to assess nuclear features and the mitotic index. Immediate use of high magnification is not recommended, because reactive tissue and inflammation, especially when macrophages are present, may be mistaken for neoplasia. For similar reasons, a definitive diagnosis may be difficult to establish with small samples or with samples that lack some normal tissue. In numerous cases, such as with osteosarcoma, mast cell tumor, transitional cell carcinoma, some soft tissue sarcomas, and squamous cell carcinoma, histologic features are sufficiently distinct to allow a definitive diagnosis on a small sample if the tumor was sampled correctly. In other cases, such as with lymphoma or granulomatous inflammation, small samples may be

TABLE 3-2 Histologic Features of Hyperplasia and of Benign and Malignant Neoplasia			
Histologic Feature	Hyperplasia	Benign Neoplasia	Malignant Neoplasia
Overall differentiation	Normal	Disorganized, but well differentiated	Disorganized, well to poorly differentiated
Cell and nuclear pleomorphism	None	Minimal	Moderate to marked
Mitotic index	Variable, usually low	Usually low	Often high
Nucleoli	Normal	Normal	Large and/or multiple
Amount of necrosis	None	Usually minimal	Minimal to abundant
Tissue demarcation	Blends with normal tissue	Expansive and/or compressive	Invasive

inadequate to establish a final diagnosis because the overall cellular pattern and interaction with normal tissue is an important diagnostic feature.

GRADING AND STAGING OF NEOPLASIA

With certain tumors, grading of the degree of malignancy is predictive of biologic behavior.[1,3,8] Grading of tumors is somewhat subjective, and reproducibility between pathologists can be variable.[11] Despite this limitation, a study of 440,000 cases of cancer in humans found that interobserver variation did not have an impact sufficient to alter the relationship between grade and outcome.[12] Another difficulty encountered during grading is tumor heterogeneity, which results in variation of features of increased malignancy from area to area. If heterogeneity is present, the most malignant areas are assessed for grading purposes. Therefore, if the sample is small, accurate grading is not possible, and results should be interpreted with caution.

Features of tumors that often are evaluated to assess the grade include (1) the degree of differentiation; (2) the mitotic index (the number of mitotic figures per 1 or 10 high-magnification × 400 fields); (3) the degree of cellular or nuclear pleomorphism; (4) the amount of necrosis; (5) invasiveness; (6) the stromal reaction; (7) the nucleolar size and number; (8) the overall cellularity; and (9) the lymphoid response. Of these factors, the mitotic index, the amount of necrosis, and the nucleolar features are the only objective features that can be quantified with manual counting, computerized morphometry, or chemical quantification.[1,3,8] Often, in the determination of a grade, individual features are scored and the scores then are added to obtain a total tumor score. The tumor scores then are separated into ranges associated with a tumor grade. Current grading scales are efficient and cost-effective and involve no new technology.

Quantifiable criteria for tumor grading have more recently been assessed with image analysis (computerized analytic morphometry). Image analysis allows for a more objective, repeatable measure, reducing interobserver variation and bias. Mast cell tumors are an example how this technique can be beneficial; in these tumors, nuclear features, such as the nuclear area, mean diameter, and perimeter, correlate with the histologic grade, which in turn is predictive of tumor biology.[13,14] Currently, computerized morphometry is not practical for routine use in diagnostic pathology because of time and effort restrictions. However, automation techniques are quickly overcoming these limitations.

The rationale for the effectiveness of tumor grades is that the histologic appearance of a tumor is an indirect assessment of molecular features (Box 3-1). For example, a correlation likely exists between tumor necrosis evaluated

Box 3-1	Molecular Features Underlying Grading Criteria

Grading criteria	Underlying molecular mechanisms
Mitotic index	Cyclins, cdks, proliferating cell nuclear antigen (PCNA), Ki-67, BrdUrd, labeling index (LI)/growth fraction (GF)
Percent necrosis	Inflammatory mediators, including eicosanoids (prostaglandins), cytokines (interleukin [IL], tumor necrosis factor alpha [TNF-α]); microvessel density (MVD)
Invasiveness	Matrix metalloproteinases (MMPs), plasminogen activators (PAs), integrin expression, cell adhesion molecules (CAMs)
Stromal reaction	Transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), MVD mediators
Nucleolar size	RNA transcriptional activity, silver staining of nucleolar organizer regions (AgNORs)
Overall cellularity	GF, apoptosis factors (i.e., FasL, caspases), tumor doubling time
Inflammatory (lymphoid) response	TNF-α; interferon gamma (IFNg); IL-2; increased MHC-II, ICAM

in many tumor grades and underlying mechanisms of angiogenesis. Inadequate neovascularity results in tumor hypoxia and thus tumor necrosis. An underlying molecular mechanism of neovascularity is vascular endothelial growth factor (VEGF) expression. Studies have shown that tumor microvessel density (MVD) and VEGF expression are strongly associated with the tumor grade and the biologic aggressiveness of the tumor.[15,16]

The tumor grade may correlate with survival, the metastatic rate, the disease-free interval, or with the frequency or speed (or both) of local recurrence. In dogs, tumors for which the grade or histologic features are predictive of biologic behavior include mast cell tumors;[17,18] lymphomas;[19,20] dermal and ocular melanomas;[21,22] mammary gland carcinomas;[23] synovial cell sarcomas;[24] multilobular osteochondrosarcomas;[25,26] hemangiosarcomas;[27] nonlymphoid, nonhematogenous sarcomas and fibrohistiocytic nodules of the spleen;[28,29] transitional cell carcinomas of the urinary bladder;[30]

squamous cell carcinomas of the tongue;[31] lung carcinomas;[32,33] appendicular osteosarcomas;[34] mandibular osteosarcomas;[35] and soft tissue sarcomas[36, 37] (Table 3-3). In humans and dogs with soft tissue sarcomas, the histologic grade is more important than the tumor subtype.[1,37,38] In cats, tumors for which the grade or histologic features are predictive of biologic behavior include lung carcinomas[33] and mammary gland carcinomas,[39] with conflicting reports regarding feline mast cell tumors and fibrosarcomas.[40-43]

Although grading systems have not been established for some malignant tumors, the pathologist often can assess presumed biologic behavior based on the overall degree of tumor differentiation. In these cases the terms *well differentiated*, *moderately differentiated*, and *poorly differentiated* may suggest a low-grade, medium-grade, and high-grade malignancy, respectively.[44] This type of assessment most commonly is done for squamous cell carcinomas, some sarcomas, and carcinomas of the mammary gland, salivary gland, gastrointestinal tract, liver, exocrine pancreas, and perianal gland. Tumor grading likely will become more widespread and important in the future, especially as novel analytic techniques and molecular tumor markers are included. The treatment applied in a particular case may also be modified based on the tumor grade; generally, more aggressive therapies are used for higher grade tumors.

The pathologist may also assist staging of the cancer by assessing tumor size, the depth of tumor invasion, the presence of tumor in regional lymph nodes, and identification of tumor in distant sites. This information is needed to stage tumors according to the TNM system (*T* stands for tumor size and/or invasion; *N* stands for nodal involvement; and *M* stands for distant metastasis).[8] For some tumors, such as bladder cancer in humans, tumor staging is based largely on depth of invasion into the bladder wall.[1,3] This criterion may prove useful in cases of bladder cancer in pets and has been shown to correlate with the tumor grade in dogs.[30] The processes of tumor grading and tumor staging are useful only if they have been shown to correlate with clinical behavior.

ASSESSMENT OF TUMOR MARGINS

Tumor margin assessment is an essential part of the pathology report whenever curative-intent surgical excision is attempted.[2] The *surgical margin* is defined as the margin beyond which the tissue remains in the surgical bed. Although seemingly simple, it can be a complicated issue considering the different routes of metastasis available to some tumors (i.e., vascular, lymphatic, bronchial, and intracavitary routes). However, assessment of the margins is critical, because it predicts the effectiveness of the prescribed surgical dose.

The completeness of surgical removal should be assessed regardless of whether the tumor is benign or malignant. Benign tumors usually are well demarcated and often surrounded by a connective tissue capsule. The margin of normal tissue around a benign, well-demarcated tumor may be less than a few millimeters thick for removal to be considered complete. Even benign tumors can recur if resection is incomplete and tumor margins are assessed as "dirty." Malignant tumors are usually invasive and not well demarcated. *Fingers* and clumps of cells may extend from the main tumor mass and invade surrounding tissue; therefore, in invasive tumors, the margins need to be larger for removal to be considered complete. If tumor cells extend to the surgical edge or within 1 mm of the edge, removal is incomplete or intracapsular, and the margins are considered incomplete. In human medicine, margins are considered close and probably incomplete if less than 1 cm of normal tissue is present around a malignant tumor.[43,45] Often this standard is not feasible in surgical oncology, because obtaining a 1 cm margin of normal tissue around a mass in some locations (e.g., on the distal extremities) is very difficult. If tumor cells do not extend to the margin, yet are within 1 cm of the margin or just outside of or on the tumor pseudocapsule, tumor margins may be considered *narrow*, *clean but close*, or *marginal*. Margins are considered *wide* if 1 to 3 cm of normal tissue is present around the tumor or if the tumor and its capsule are not entered. Margins are *radical* if more than 3 cm of normal tissue is present around the tumor or if the entire compartment or structure is removed, as in amputation.[45,46]

In light of this vague terminology, specifics on the margins should be given to reduce ambiguity. These should include numeric measurements of the amount of normal tissue at the surgical margin and the type of tissue present. Not all normal tissues are equal barriers to tumor invasion; fascial planes, tendons, cartilage, and bone are more resistant to tumor invasion than is fibrofatty tissue. An additional consideration is tissue artifactual change and shrinkage during formalin fixation and tissue processing. If tissue samples are fixed in containers that are too small or are irregularly shaped, or if the samples are handled aggressively during the biopsy procedure, the shape and size of the tissue can be altered dramatically during fixation, making identification and interpretation of margins difficult.[47] Tissues also vary with regard to the amount of fixation- and processing-induced shrinkage artifact.[48,49] As the normal tissue at the margins shrinks, interpretation of the amount of tissue at the margin can be compromised. Few studies have critically evaluated the amount of shrinkage artifact in animal tissues, but loose, fatty tissue often shrinks by at least 50% with fixation. In cases involving narrow tumor margins, options include a closer monitoring protocol for recurrence; immediate, more aggressive

TABLE 3-3	Neoplasia with Grades or Histologic Features Having Prognostic Significance		
Tumor Type	**Grades**	**Features of Importance**	**Reference**
Mast cell tumor (dog)	I to III	Cellularity, nuclear to cytoplasmic ratio, cell morphology, mitotic index, depth, necrosis, granularity	17, 18
Mast cell tumor (cat)	Well or poorly differentiated, histiocytic	Cellular and nuclear pleomorphism, mitotic index	40, 42
Lymphoma	Low, intermediate, high	Architecture, mitotic index, nuclear size, morphology, T/B immunophenotype	19, 20
Dermal melanoma	Well or poorly differentiated	Mitotic index	21
Anterior uveal melanoma (cat, dog)	I to VI (or early, moderate, or advanced); benign, malignant (dog)	Mitotic index, extent of invasion	22, 85
Soft tissue sarcoma (dog)	1 to 3; or mitotic index >9,* mitotic index <9*	Overall differentiation, mitotic index, necrosis	36, 37
Lung/pulmonary carcinoma (dog)	1 to 3	Overall differentiation, nuclear pleomorphism, mitotic index, necrosis, nucleolar size, fibrosis, invasion	32, 33
Lung/pulmonary carcinoma (cat)	Moderately or poorly differentiated	Organization, pleomorphism, pulmonary and vascular invasion	33
Mammary gland carcinoma (dog)	Well, moderately, or poorly differentiated	Invasion, nuclear differentiation, lymphoid response	23
Mammary gland carcinoma (cat)	Well, moderately, or poorly differentiated	Differentiation, cellular pleomorphism, mitotic index	39
Synovial cell sarcoma	1 to 3	Nuclear pleomorphism, mitotic index, necrosis	24
Primary and metastatic osteosarcoma (dog)	I to III	Tumor pleomorphism, mitoses, tumor matrix, cell density, necrosis, vascular invasion	34
Multilobular osteochondrosarcoma	1 to 3	Borders, lobule size, organization, mitotic index, nuclear pleomorphism, necrosis	25, 26
Mandibular osteosarcoma	1 to 3	Nuclear pleomorphism, mitotic index, necrosis	35
Hemangiosarcoma	1 to 3	Overall differentiation, nuclear pleomorphism, mitotic index, necrosis	27
Nonlymphoid, nonangiomatous splenic sarcomas	Mitotic index 0-9,* mitotic index >9*	Mitotic index	28
Fibrohistiocytic nodules of the spleen	1 to 3	Proportion of lymphocytes and multinucleated cells, mitotic index	29
Transitional cell carcinoma	1 to 3	Cytoplasmic and nuclear variation, nuclear placement, nucleolar size and number, mitoses	30
Squamous cell carcinoma	1 to 3	Overall differentiation, mitotic index, nuclear pleomorphism, invasion, stromal reaction	31

Sum of mitoses in 10 × 400 fields.

surgical removal; and/or the application of adjuvant therapy (e.g., radiation therapy).

When the surgeon excises a tumor, the surgical margins should be marked with a suture, if a specific area of concern is present, or with india ink or commercially available margin dyes.[2,50] A disadvantage of the suture method is that sutures must be removed before tissue is sectioned on the microtome to prevent sectioning arti-facts. Ink is retained through processing of the tissue, permitting the pathologist to visualize the inked margin through the microscope. An inked margin also prevents the pathologist from mistaking false margins created by tissue trimming or retraction of fatty or loose connective tissue from the tumor mass. (In some cases, specimens can be pinned to cardboard to prevent or reduce retrac-tion caused by formalin fixation.) Furthermore, inked margins allow for distinction of artifactually created margins that may occur during processing and section-ing on the microtome if the tissue section tears or frag-ments. Inks come in a variety of colors, permitting more than one margin to be inked for proper orientation into cranial, caudal, superficial, deep, medial, or lateral planes.[50] Black and yellow inks show especially well.

If the tumor is not marked, a standard tissue section often is taken in one complete plane, including tumor and surrounding normal tissue. Two samples are then taken perpendicular to the original section, one on either side. Margins that appear to be close to the tumor also may be sampled. This method allows assessment of four lateral margins, the deep margins, and the superfi-cial margin if relevant.[51] Standardization among labora-tories of assessment of margins and trimming of tissues has not been accomplished in human or veterinary diagnostics, therefore direct comparisons of published studies are difficult. Margins in cavities or air spaces are not considered true margins, because no tissue interface exists. Margins around mast cell tumors are of special concern in veterinary medicine. These tumors frequently have nests of cells that are separated from the main mass by normal tissue. Even after microscopic evalua-tion, distant nests may be present that were not recog-nized in the planes sectioned. For these reasons, a tumor's known biologic behavior should always be considered with the histologic assessment of surgical margins when the need for additional surgery, ancillary treatment, or periodic rechecks is determined. For mast cell tumors, a 2 cm margin of grossly normal-appearing tissue in all lateral directions and one complete tissue plane deep is usually recommended.[52] Mast cell tumors pose an additional problem; distinguishing a resident, nonneoplastic mast cell from a well-differentiated neoplastic cell is difficult or impossible.

Soft tissue sarcomas are another common tumor in veterinary medicine that require special attention to margins. To the surgeon, these tumors often appear well demarcated and easily shell out. However, fine strands and invasive fingers of neoplastic cells often extend to and beyond the margins of grossly normal-appearing tissue. At least 3 cm of grossly normal-appearing tissue should be included in the resection, if possible.[37,53] If margins are not complete, the surgeon may wish to further resect the suture line and wound bed to prevent tumor recurrence. In these resected samples, small foci of tumor may be obscured within the granu-lation tissue and inflammation produced by the first surgery. However, the deep and lateral margins adjacent to the suture line usually can be assessed for the presence of residual tumor and the effectiveness of the surgical revision. Inability to identify tumor cells in resected suture areas should not be interpreted to mean that the second surgery was unnecessary; rather, it reflects the difficulty of identifying microscopic foci of neoplastic tissue amidst the inflammatory and reac-tive tissue.

If a surgical resection is determined to be complete by microscopic evaluation, the chance of local recur-rence is reduced, but by no means is local tumor control guaranteed. Recurrence of soft tissue sarcomas in humans has been shown to occur in about 10% of cases in which margins were deemed complete[54]; a similar situation is likely in veterinary medicine. Serial sections through every margin of a specimen are not feasible; consequently, tumor extension to the cut edge may be missed. If the surgical margin is incomplete, especially in a well-inked sample, local recurrence is almost guaranteed unless further treatment is initiated.

ASSESSMENT OF TREATMENT RESPONSE

Sometimes histologic assessment of the preoperative treatment response may help predict the outcome and could even affect subsequent therapy. Assessment of preoperative therapy is most often done with osteosar-comas and soft tissue sarcomas. *Percent tumor necrosis* is the parameter most commonly used to quantify the impact of presurgical chemotherapy or radiation therapy. In dogs with osteosarcomas, the percent tumor necrosis is a good predictor of local recurrence after limb-sparing surgery that follows neoadjuvant radiation therapy and/or chemotherapy. In some studies, tumor necrosis of 90% or greater correlated with a local control rate of 91%; tumor necrosis of 80% to 90% correlated with a local control rate of 78%; and tumor necrosis of less than 80% correlated with a local control rate of only 28%.[55,56] In humans, the percent tumor necrosis in osteosarcoma after preoperative chemotherapy is also predictive of survival, and individuals who have a poor histologic response may be treated with more aggressive

alternative chemotherapeutic regimens.[57] In soft tissue sarcomas, the percent tumor necrosis has been used to assess presurgical therapy in humans,[58] and this also could be done in veterinary patients.[58]

The effect of previous treatment (i.e., radiation, chemotherapy, or photodynamic therapy) may also be evaluated histologically when progressive growth of tissue in an area occurs. In these cases, distinguishing between reactive tissue and neoplasia is important but also extremely difficult. Inflammation, fibrovascular proliferation (granulation tissue), or epithelial hyperplasia (if applicable) usually is present in the pretreated area. Furthermore, especially after radiation therapy, the area may have some bizarre reactive cells, including fibroblasts (called *radiation atypical fibroblasts*), with many features of malignancy.[59] If tumor cells are identified, the clinician may want to know whether these cells are viable or dead, or viable but sterilized (incapable of reproduction) by radiation or chemotherapy. Distinguishing between a viable cell and a dead cell often is possible, but determining whether a cell is viable but sterilized, or nonclonogenic, is not possible microscopically. However, the presence of numerous mitotic figures in a viable-appearing tumor suggests active regrowth. Some immunohistochemical and histochemical techniques that identify markers of proliferation in tumor cells (i.e., proliferating cell nuclear antigen [PCNA], Ki-67 growth factors, and silver staining of nucleolar organizer regions [AgNORs]) theoretically could help determine the proliferative capability of pretreated tissues.

Molecular diagnostic techniques (see Chapter 8) hold promise for identifying the adequacy of treatment or the treatment response, or both. The use of PCR to identify circulating lymphoma cells in dogs during clinical remission holds promise as an early indicator of tumor recurrence, before disease is clinically, histologically, or cytologically identifiable.[60] New techniques that identify large portions of the tumor's genomic expression (gene expression microarrays) and proteomic expression (tissue microarrays, protein microarrays, and mass spectrometry) can identify tumor gene and protein profiles that are predictive of a tumor's biologic behavior and its response to treatment or of early tumor recurrence.

SPECIAL PROCEDURES

Approximately 90% of oncology cases in humans can be diagnosed by light microscopy using haematoxylin and eosin stains.[2] This percentage also is probably a close estimation of the situation in veterinary medicine. In the remaining 10% of cases, special stains or procedures, such as immunohistochemistry or electron microscopy, may help. Immunohistochemistry, flow cytometry, or other molecular techniques may also be

useful in predicting tumor behavior or differentiating benign from malignant tumors.

Special Stains

Special stains commonly are used to assist the diagnosis of certain poorly differentiated tumors.[8] The special stains used most often are toluidine blue and Giemsa, which are used to identify granules in poorly differentiated mast cell tumors. Some feline and ferret mast cell tumors stain better with periodic acid–Schiff (PAS) than with toluidine blue.

Masson's trichrome or other trichrome stains may be used to identify collagen fibrils; this can help differentiate certain mesenchymal tumors, such as those derived from muscle (leiomyomas, leiomyosarcomas, rhabdomyoma, rhabdomyosarcomas), and those that produce matrix (fibromas, fibrosarcomas). It is important to keep in mind, however, that muscle-derived tumors may have a small amount of collagen, and fibrosarcomas can be so poorly differentiated that little collagen is produced. Alcian blue stain may help identify ground substance glycosaminoglycans that may be seen in some neurofibrosarcomas or myxosarcomas. Mucicarmine stain or PAS is useful in mucosal tissues for identifying poorly differentiated carcinomas. A melanin bleach or iron stain may help distinguish between hemosiderin and melanin in suspected cases of melanoma. Other, less frequently used special stains, such as reticulum fiber stain, also are available.

Newer techniques, such as silver staining of nucleolar organizer regions (AgNORs), are available and have been shown to be prognostically predictive in canine malignant lymphoma[61] and mast cell tumors.[62]

Immunohistochemistry

Immunohistochemistry (IHC) can aid the classification of several tumors in veterinary medicine and is a widely used diagnostic technique. IHC is a staining procedure that uses antibodies to identify specific cellular and extracellular molecules *ex vivo*, such as cytoplasmic intermediate filaments, secretory substances, and cellular proteins. IHC can be done on frozen sections or specimens routinely fixed in formalin and processed into paraffin blocks. The tissue sections are incubated with primary antibodies to specific cell components (the antigens). These sections with bound primary antibody are then exposed to secondary antibodies directed against the primary antibody. The secondary antibodies are linked to peroxidase or avidin-biotin peroxidase complexes. The peroxidase catalyzes a reaction in the presence of dye that precipitates at the site of the complex and is visible with light microscopy.[2] As an alternative, alkaline phosphatase enzyme systems are also available.

Commonly used IHC stains are those for intermediate filaments, such as vimentin for mesenchymal cells, cytokeratin for epithelial cells, and desmin or actin for muscle cells.[2,63-65]

IHC also can be used to identify certain hormonal secretory products (e.g., insulin, glucagon, thyroglobulin, and calcitonin) to accurately identify endocrine tumors.[2,66,67] Other proteins and secretory products that may be stained include factor VIII–related antigen (von Willebrand's factor) or CD31 platelet-endothelial cell adhesion molecule (PECAM) for endothelial origin tumors; S-100 protein, HMB-45, melan A, and tyrosinase-related protein-2 (TRP-2) for melanomas; [68-70] chromogranin A and synaptophysin for neuroendocrine tumors; neuron-specific enolase (NSE) for nervous tissue or neuroendocrine tumors; and glial fibrillary acidic protein (GFAP) for astrocytic tumors. IHC stains for macrophages or histiocytes include alpha-1-antitrypsin or lysozyme. T- and B-cell markers, such as CD3 and CD79a, respectively, are used to immunophenotype and classify malignant lymphoma in dogs and have also been shown to be of prognostic significance; generally, T-cell lymphoma has a poorer prognosis than B-cell lymphoma in dogs.[20,61] IHC is also useful for determining the cell proliferative rate; this can be done using Ki-67 and PCNA staining, markers of multidrug resistance (e.g., P-glycoprotein),[71] or altered oncogenes such as p53, c-Kit, p21, Rb and PTEN.[72-74]

Although IHC can be a valuable tool, some complicating factors exist. A negative stain does not exclude a certain cell type, because technical difficulties or poor tumor cell differentiation may produce a negative stain. The most common technical problem that causes negative staining is extended fixation in formalin, which results in excessive cross-linking of the antigenic components or loss of soluble proteins into the fixative. Protein cross-linking that masks some antigens often can be "unmasked" by pretreating sections with trypsin or pepsin or by using heat-induced epitope retrieval techniques (HIERs). Areas of tissue necrosis, autolysis, section drying, hemorrhage, and sometimes collagenous matrix components can cause excessive nonspecific background staining. A skilled pathologist who is familiar with the IHC stain should be asked to differentiate background stain from tumor-specific stain and to navigate technical difficulties. IHC does not always distinguish between neoplastic and nonneoplastic tissue, because reactive tissue and tumor cells may have a similar cellular protein expression. For example, IHC does not distinguish between epithelial hyperplasia and carcinoma, because both of these may be positive for cytokeratin.

Considerable cross-reactivity of staining in different tumor types may occur because some markers may be found in a variety of cells or tumors, (e.g., S-100) in melanomas, cartilage, and certain epithelial cells.[2,75] Because most tumor markers have limitations, the best and most reliable results may be obtained by using a panel of IHC stains rather than relying on a single stain.

Electron Microscopy

Electron microscopy (EM) involves preserving very small, representative tumor samples in a special fixative (e.g., glutaraldehyde), processing tissue into epoxy-based plastic blocks, and sectioning the blocks into 1 μm thick slices to determine the adequacy of the sample and inclusion of appropriate tumor cells. Subsequently, sectioning is done at about 600 Å; the sections then are stained with a heavy metal–based stain and examined with the electron microscope. Samples fixed in formalin can be used, although the quality of the subsequent sections is less than ideal. EM may help identify certain specific features, such as intercellular junctions or basal lamina in epithelial cells, melanosomes in melanocytic cells, mast cell granules in mast cells, neurosecretory granules in neuroendocrine cells, or mucin droplets in certain epithelial cells. These features are useful for distinguishing carcinomas from lymphomas and for identifying melanomas, mast cell tumors, and neuroendocrine tumors, respectively. However, unless a specific feature is sought, EM is no more useful than higher magnification of a tumor that could not be diagnosed with the light microscope. Furthermore, EM is not useful for distinguishing benign from malignant cells in many cases, because the magnification is too high and the tumor pattern in the tissue is not evident.[3,8] Not all veterinary diagnostic laboratories have the technical support and equipment needed for EM.

Flow Cytometry and Polymerase Chain Reaction

Flow cytometry is an analytic procedure that can be used to evaluate cell suspensions obtained from suspected neoplastic masses. In human medicine, this procedure frequently is used to diagnose and occasionally to monitor for recurrence of various tumors. It is especially useful for detecting neoplastic cells in the urine of bladder cancer patients and in the evaluation of cell suspensions in cases of suspected leukemia and lymphoma. With solid tumors, the cells first must be disassociated to create a single cell suspension. Cell suspensions are stained with specific fluorochromes, passed through the flow cytometer chamber, and analyzed and sorted using a focused laser beam. The most routine analysis is determination of the DNA content or ploidy of the cells. Malignant cells may be diploid (normal DNA content) or aneuploid (nondiploid); normal tissue, benign tumors, and reactive tissues usually are diploid. Occasionally, however, benign tumors and reactive tissue can be aneuploid. In some cases, aneuploidy may be prognostically predictive. Flow cytometry also can be used to evaluate

the S-phase distribution or cell cycle time if the tumor is sampled at appropriate times after the patient has been injected with bromodioxyuridine.[2,8]

Flow cytometry has been used to evaluate tumors in dogs, although it has yet to become a routine procedure. In the earliest report, various canine tumors were characterized for DNA ploidy.[76] Tumor cell heterogeneity, comparisons of primary and metastatic tumors, and the positive predictive value of kinetic parameters in canine osteosarcomas have also been evaluated with flow cytometry.[77,78] In addition, it has been shown to be predictive of the behavior of mammary gland tumors,[79] melanomas,[80] and plasmacytomas.[81] Flow cytometry can be useful for analyzing abnormal populations of white blood cells in blood or fluid, which helps to distinguish lymphoma from reactive processes. As flow cytometry samples cell suspensions derived from tumor masses, a correlate sample from the same site or same specimen should always be taken for histopathologic assessment. Histologic correlation is necessary, because flow cytometry cannot distinguish a benign from a malignant diploid tumor, nor can it always identify the tumor type.[2] Currently the use of flow cytometry in the analysis of cancer (excluding leukemia of bone marrow origin) in veterinary medicine is mostly investigational, but the technique undoubtedly will be used more routinely in the near future.

PCR can be used to distinguish malignant lymphoma from reactive processes. PCR amplifies the DNA encoding the antigen-binding region of lymphocytes; a clonal or single-size product indicates malignancy. In an early study, 91% of lymphomas were identified by this technique.[5] PCR also can identify the B- or T-cell immunophenotype of the lymphoma. Currently, air-dried aspirates and fresh or formalin-fixed tissue can be used for this technique.

CLINICAL-PATHOLOGIC CORRELATION AND SECOND OPINIONS

Sometimes the pathologist cannot make an accurate diagnosis without clinical correlations.[1,3,8] This is especially true for some primary bone tumors and for secondary tumors involving bone. Diagnosis of a surface or juxtacortical osteosarcoma is based on both radiographic and histologic features. An osteoma may be difficult to distinguish from reactive bone without a corroborative radiograph. A synovial cell sarcoma may be difficult to distinguish from other sarcomas or even inflammatory or immune-mediated joint disease without radiographic or gross evidence of joint involvement and bone invasion. An acanthomatous epulis may be difficult to distinguish from a fibromatous epulis unless bone invasion is present in the former, and such invasion cannot be identified without appropriately deep biopsy samples that include

underlying bone. The best example of the need for clinical and pathologic correlation is the histologically low-grade yet biologically high-grade fibrosarcoma of the canine head, in which the histologic appearance is that of benign fibrous tissue but the clinical presentation is that of an aggressive invasive mass.[9] These examples demonstrate the importance of supplying the pathologist with an accurate history, with pertinent clinical results, in addition to the biopsy sample. In some cases, photographs of the tumor site or inclusion of radiographs or radiographic findings can be most helpful.

Before any major treatment is undertaken, or *if a pathology diagnosis is not consistent with the clinical presentation*, a second opinion should be requested from the pathologist. In physician-based medicine, a review of second opinion surgical pathology at major hospitals revealed that 1.4% to 5.8% of reviews produced major changes in diagnosis that resulted in modification of therapy or prognoses. The researchers concluded that despite the extra cost, mandatory second opinions should be obtained whenever a major therapeutic endeavor is considered or if treatment decisions are based primarily on the pathology diagnosis.[82-84]

The two major categories of errors that may occur are technical errors and errors in interpretation of the tissue.[44] Technical errors may occur if the histotechnologist improperly labels specimens, tissue blocks, or slides or fails to process all the critical tissue submitted by the clinician. If tissue is improperly processed because of equipment malfunction or because it was poorly sectioned, artifacts can occur that make the tissue specimen impossible to interpret. Errors in interpretation by the pathologist may occur in difficult cases. If a pathology service staffed by physicians is used for veterinary samples, certain tumors, such as histiocytomas, mast cell tumors, transmissible venereal tumors, and perianal gland adenomas, may be misdiagnosed, because these do not have a human counterpart. Many pathologists obtain opinions from other pathologists when confronted with difficult cases, just as clinicians seek second opinions on difficult radiographs or clinical problems. *The clinician should never hesitate to ask for a second opinion, nor should the pathologist be offended by the request.* Each pathologist approaches a section differently, and in some cases one approach might prove more accurate than another. A second or even third pathologist can offer a different perspective on a difficult case, offer an alternative diagnosis, confirm the primary pathologist's diagnosis, or confirm that an accurate diagnosis is not possible. Because the patient's treatment options or decisions regarding euthanasia often are based on the final pathology diagnosis, it is not at all unreasonable for the clinician to request a second opinion. A misdiagnosis can result in costly, ineffective, and untimely treatments that can cause undo discomfort for patients. They can

result in unnecessary surgery; unnecessary chemotherapy or radiation therapy; insufficient treatment, resulting in cancer progression; and, worst of all, unwarranted euthanasia. Given these possible scenarios, second opinions are not only prudent but highly recommended.

The clinician must have a knowledge of the pathology of neoplasia to understand neoplastic conditions and the limitations of histopathologic assessment in the diagnosis of neoplasia. In the case of tumor diagnosis, histopathologic assessment of a thin slice of tissue may not always be an exact science. The pathologist and the clinician must work together to establish the most appropriate diagnosis so that proper treatment can be initiated.

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The Epidemiology and Incidence of Cancer

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There is good evidence to support the statement that cancer is an important disease of dogs and cats at all ages and the leading cause of death in older pet animals. However, developing precise estimates of cancer morbidity and mortality rates in pet animals has been difficult to achieve. When compared to human cancer epidemiology, conducting epidemiologic studies of cancer in dogs and cats is limited by three key data gaps. First, the lack of a census for pet animals precludes measuring morbidity and mortality rates directly, since the population from which the cases originate is generally unknown. In 2001, there were an estimated 73.9 million dogs and 90.5 million cats living in all households in the United States.[1] Over 63% of all households reported owning at least one pet during 2005. Eighty-five percent of dog-owning households reported taking their dog to a veterinarian in that year, with an average of 1.8 visits per dog. Second, case ascertainment is incomplete and subject to various forms of bias. Since the diagnosis of cancer may require special procedures and diagnostic tests including radiography, ultrasound, and biopsy, not all cases of cancer that are presented to veterinary practices are diagnosed equally. Further, because of the relatively high costs for many advanced diagnostic procedures and treatments, some owners may elect to euthanize pets, especially older animals, without a biopsy or a definitive diagnosis. Third, since death certificates are not required, estimation of mortality has been restricted to relatively few studies where suitable population denominators existed. Similarly, to calculate incidence rates one needs data about the population from which the cases are drawn. For human epidemiology, the creation of state-based cancer registries with comprehensive, mandatory reporting from physicians, hospitals, and diagnostic laboratories has permitted the calculation of population-based incidence rates using census data in denominators. Cancer registries are an invaluable source of data for measuring risk and for comparing cancer incidence over time and from place to place. Most studies of the epidemiology of cancer in pets have been forced to rely on surrogate data sources to estimate morbidity and mortality rates, since population-based data are not readily available.

DESCRIPTIVE EPIDEMIOLOGY

Descriptive epidemiology is the first phase of investigation in which disease is described according to three features: the person or animal affected, time, and place. Descriptive epidemiology is used to generate hypotheses regarding potential associations with a variety of exposures, some of which may lie in the causal pathway to disease. Therefore, descriptive studies are essential for understanding the occurrence of cancer in pet animals and setting the stage for testing hypotheses regarding potential causal factors. Hypothesis testing will be discussed later in this chapter under the heading Analytic Epidemiology. Understanding the risk factors for cancer is critical for developing preventive strategies aimed at reducing the risk of developing cancer. Cancer prevention is at its infancy in veterinary medicine and has had only limited success in human medicine. Screening programs for high-risk individuals in human and veterinary oncology may identify cancer at an early stage when the likelihood of treatment success is highest.

Rates of disease are calculated to describe the occurrence of cancer in animal or human populations. Morbidity rates estimate the number of new cases of cancer in the population (incidence rates) or existing cases of cancer in the population (prevalence rates), while mortality rates describe deaths from cancer in the population over a suitable time period.

MORBIDITY RATES

Cancer Incidence Rates

The incidence rate (sometimes referred to incorrectly as "incidence") of cancer is measured as the number of *new* cases of cancer developing in a defined

population per unit of time such as 1 year. Age-, sex-, and breed-specific incidence rates provide detailed information about the occurrence of cancer in specific segments of the population and are important elements of descriptive epidemiology. Unfortunately, incidence rates for canine and feline cancer are difficult to measure since population-based registries do not exist. Despite this limitation, several studies have been able to calculate incidence rates for canine and feline cancers.

The Alameda and Contra Costa Counties Animal Neoplasm Registry

The most comprehensive effort to estimate cancer incidence rates was conducted in a survey of veterinary practices in Alameda and Contra Costa counties (in California) from 1963 to 1966. Dorn et al. attempted to identify all neoplasms diagnosed by enlisting the cooperation of 65 veterinary practices in the two Bay Area counties and 11 practices in contiguous counties that treated animals from Alameda and Contra Costa counties.[2] The investigators encouraged participating veterinarians to submit tissue from all suspected cases of neoplasia in return for a free histologic diagnosis. The denominator was estimated by conducting a survey in a probability sample of households in Alameda County to derive the age, sex, and breed distribution of pets and to determine whether the household had used veterinary services. A denominator of dogs and cats from "veterinary using households" was created for the calculation of incidence rates. An estimated annual incidence rate of 3.8 cases of cancer per 1000 dogs was calculated (381/100,000), indicating that almost 4 dogs per 1000 living in households that used veterinary services have a newly diagnosed case of cancer each year. The incidence rate for all cancer in cats was 155.8/100,000. Although this study is badly outdated and many limitations of the data exist due to incomplete ascertainment associated with the diagnostic practices in use in the 1960s and changes in breed distribution since publication, it remains the seminal study for estimating the incidence of canine and feline cancer.[3]

The Tulsa Registry of Canine and Feline Neoplasms

The Tulsa Registry of Canine and Feline Neoplasms was the second animal tumor registry in the United States.[4] Histologically confirmed tumors were included in the numerators for calculations of total incidence rate, for rates for benign and malignant neoplasms, and for specific cancer incidence rates. The population at risk was derived by counting all animals seen at all of the 35 participating animal hospitals during the first 2 registry years to estimate the "veterinary-using" population. Among the 63,504 dogs seen by participating veterinarians during the first year, 715 had one or more tumors for an incidence rate of 1126 per 100,000. Among 11,909 cats, 56 had at least one tumor for an incidence of 470 cases per 100,000. Tumors of the skin were the most frequently diagnosed neoplasms, as was the case for the California registry study. Note that these rates are about three times higher than those reported for California, due mainly to differences in the method of estimating the denominator.

Studies of Insured Dogs

Several studies of morbidity and mortality in insured dogs in Sweden have been published.[5,6,7] Data from these studies are derived from observations on more than 200,000 dogs insured by one Swedish company and are believed to represent approximately one third of all Swedish dogs.[5] Approximately 50% of Swedish dogs are insured for veterinary care, and approximately two thirds of these are insured by a single company.[6] Dogs were insured for life insurance or for veterinary care. Unfortunately from the standpoint of cancer epidemiology, dogs were only eligible for life insurance initially up to the age of 10 years[5] and more recently until age 11.[7] Few older dogs are covered for veterinary care. Morbidity rates were based on accessions for veterinary care that exceeded a deductible sum (approximately $100). It is not known to what extent insured dogs represent the total population of dogs. Despite these limitations, this database allowed the investigators to calculate overall morbidity and mortality rates for cancer in dogs as well as age-, sex-, and breed-specific rates. The risk for morbidity due to neoplasia was 2.66% among females, 1.58% among males, and 2.13% overall.[7] In one publication, the incidence rate of mammary tumors was determined to be 111 per 10,000 dog-years at risk (DYAR).[8] The mortality rate for mammary tumors was six deaths per 10,000 DYAR. High-risk breeds (e.g., English springer spaniel, Doberman pinscher, boxer) were identified from the Swedish insurance data. The rates were not adjusted for neuter status but most female dogs in Sweden are not neutered.[8]

Studies of Military Working Dogs

The population of working dogs maintained by the U.S. Department of Defense has also provided an opportunity to study the lifetime incidence of neoplasia and causes of death.[9] Approximately 1700 working dogs make up this cohort, which consists primarily of Belgian shepherd dogs (61.5%) and German shepherd dogs (30.6%).[10] Among this population of primarily intact male dogs, the lifetime incidence of neoplasia exceeded 30%, with 10% developing more than one neoplasm. Seminoma of the testicle was the most frequently occurring malignancy.[9] Although the data are restricted mainly to two breeds, the extensive medical surveillance and necropsy examination that are applied routinely make this dataset of interest.

Cancer Prevalence Rates

Prevalence is the proportion of *existing* cases of cancer in a population at a single point in time (point prevalence) or over a period of time such as 1 year (period prevalence). Cancer prevalence has been estimated for canine and feline cancers using several types of populations as the denominator. Prevalence estimates have been based on necropsy series,[11,12] diagnostic laboratory accessions,[13] examination of dogs from pounds,[14] single veterinary teaching hospitals,[15] a group of veterinary teaching hospitals[16] and surveys of breed club members (Bernese Mountain Dog Club, Golden Retriever Foundation)[17,18] and dog owners.[19] It is critical to consider the population denominator from which the cases of cancer were identified in order to understand the strengths and limitations of the data. None of the populations described is population-based. The denominators used do not represent the population of dogs or cats at large; therefore, estimates of prevalence obtained from these data are subject to selection bias. Nonetheless, usable information can be gleaned from these sources that permit estimation of prevalence adequate to describe general features of the distribution of canine and feline cancer. For example, the high risk of cancer in the boxer dog was first identified in 1959 by examining two case series of malignant lymphoma obtained from 130 participating veterinarians and dog registration data.[20] These findings were confirmed in a series of diagnostic laboratory accessions,[13] by examining data from the University of Pennsylvania veterinary teaching hospital,[15] and with data from the Veterinary Medical Data Program and a series of hospital accessions.[21] Boxer dogs were also identified as a high-risk breed for malignant lymphoma in the Alameda and Contra Costa counties animal neoplasm registry.[22]

The Veterinary Medical Data Base (VMDB)

The VMDB was initiated in 1964 by the National Cancer Institute for the purpose of studying cancer in animals. The program was based on collecting standardized abstracts of descriptive, diagnostic, and surgical data from the records of animals seen at participating North American veterinary teaching hospitals. The program began with the recruitment of 11 hospitals and now includes 26 universities that have collectively submitted more than 7 million records to this database. Data from the VMDB have been used for numerous descriptive and analytical epidemiological studies. These investigations have been limited by the small number of potential risk factors included on the abstract but have been useful for identifying risks for numerous types of cancer associated with age, sex, breed, and neutering status. The data have been used rarely to examine geographic differences in cancer prevalence.[23] Lack of data for diet, residential history, and specific environmental exposures has precluded the exploration of specific etiologic hypotheses. However, an extensive series of papers published with VMDB data has permitted the characterization of cancer risk by age, sex, and breed for many canine and feline cancers including tumors of the bone,[24] lymphatic system,[21] skin,[25] pancreas,[26] nervous system,[28] thyroid,[27] mouth and pharynx,[29] bladder,[30] testicle,[31] nasal cavity and paranasal sinuses,[32] kidney,[33] ovary,[34] and mammary gland.[35] Despite changes in the distribution of specific breeds of dogs and cats, neutering practices, and longevity over time, data from these studies remain the basis of our understanding of the descriptive epidemiology of many canine and feline tumors. The fundamental weakness in these data is their hospital-based nature and the potential for the introduction of selection bias from nonrepresentative numerator and denominator data.

Survey Data

Several surveys of animal owners have been published that provide a crude estimate of the prevalence of various disorders and causes of death. Danish investigators queried members of the Danish Kennel Club in 1997 and received information for 4295 dogs.[36] The kennel club represents approximately 53% of all registered dogs in Denmark where registration is compulsory. Tumors accounted for 4.4% of all reported diseases. Higher breed-specific prevalence rates of neoplasia were reported for the English springer spaniel, Hovawart, Samoyed, flat-coated retriever, golden retriever, and beagle, but specific causes of neoplasia were not identified. These authors also described owner-reported causes of mortality for 2928 dogs from the same source population.[37] The proportionate cancer mortality was 14.5% (cancer deaths divided by all causes of death). High prevalence of death due to cancer was reported for the Bernese mountain dog, flatcoated retriever, and beagle. Since the data from these studies are self-reported and not confirmed by medical record validation, they are of limited usefulness, despite having a population-based origin.

In the United States, several surveys of breed club members have been undertaken. The Golden Retriever Club of America conducted a national health survey of its membership in 1998 and described the findings for more than 1440 dogs.[18] The lifetime risk of a golden retriever developing a neoplasm was 50%. Hemangiosarcoma was the most frequently reported cancer with a lifetime risk of 1 in 5, followed by malignant lymphoma with a lifetime risk of 1 in 8. This study separated diagnoses confirmed by a veterinarian from those reported by the owner only. Members of the Bernese Mountain Dog Club confirmed the importance of cancer in this breed in a survey.[17] Cancer deaths represented 49% of 261 deaths. Malignant histiocytosis was the most frequent type of cancer in this breed, confirming earlier published reports.[38] Finally, Mark Morris Associates conducted an animal health survey in 1998 by mail-back questionnaire

in its newsletter. Despite a poor response rate (2.7%), 2003 responses were received. Cancer was listed as the leading cause of death among dogs (47%) and cats (32%).[19]

MORTALITY RATES

Cancer Proportional Mortality Rates

Mortality from cancer in dogs and cats has been reported infrequently. The most common source of mortality data is the cancer proportional mortality rate (CPMR). In calculating the CPMR, the number of animals that died from cancer is divided by the number dying of all causes. Typically such data are found in series of necropsied cases. The main shortcoming from such analyses is that the proportion depends not only on the frequency of cancer as a cause of death but on the frequency of all other disorders in the database. Proportionate mortality analyses are prone to selection bias due to the patterns of referral of the institution, the probability that a necropsy will be performed, special interests of the staff, and so on. Many early descriptions of cancer in pet animals were based on series of necropsy examinations and used to infer data for incidence rates with obvious limitations.[11,12] A study of 2002 dogs necropsied at the Angell Memorial Animal Hospital found that cancer accounted for 20% of the deaths at 5 years and increased to over 40% in dogs 10 years of age and older, thus supporting the contention that cancer is the leading cause of death among older dogs.[39] Another study assessed the CPMR among 1206 golden retrievers, boxers, German shepherd dogs, Labrador retrievers and rottweilers.[40] The CPMRs for golden retrievers and boxers were significantly higher than those for the other breeds. The highest CPMR was found for golden retrievers (56.6%), followed by the boxer (51.9%), and German shepherd dog (36.7%). The lowest CPMR was for the rottweiler (28.2%). These data illustrate the importance of cancer as a cause of death even among lower-risk breeds. The age at death was not significantly different among the five breeds, reducing the possibility that confounding by age played an important role in the findings.

Ideally, mortality data should be obtained from longitudinal, prospective studies of dog populations or representative samples of these populations. As cohort studies become more commonplace in pet animals, valid mortality data may become available. One potential source of such data is a national database representative of dogs examined by small animal veterinarians and reported systematically by electronic communication.[41] The potential usefulness of such a system has been validated by a study assessing the frequency of postvaccinal reactions and vaccine site-associated sarcomas in cats.[42] Data from large populations of dogs identified through

a national surveillance system represent an additional potential source of mortality data.[43]

Finally, clinical descriptions of death rates among dogs with cancer (dogs dieing of cancer/dogs with cancer) are often referred to as "mortality" rates. These rates are more properly referred to as "case fatality rates" but the usage has become widely accepted.

Adjusted Rates

When rates are compared between breeds, sexes, neuter status, geographic location, and other factors, it is important to consider the possibility that confounding may be responsible for observed differences. The risk of most cancers is related to age. Therefore, differences in the age distribution between groups of dogs may introduce bias due to confounding. Standard methods exist for adjusting rates for other potential risk factors such as age.[44] Studies of mammary cancer that fail to adjust for neuter status are also subject to bias from confounding.

CANINE AND FELINE CANCER INCIDENCE RATES

As described earlier, data for incidence rates of cancer in dogs and cats are limited to a few sources, most of which are badly outdated. The single most important source of incidence data remains the California Animal Neoplasm Registry,[2,3] and it is widely referred to when incidence rates are described, despite the fact that the data are more than 40 years old. Table 4-1 provides incidence data for canine and feline cancer obtained from several publications as well as incidence data for human cancer in Alameda County for the same time period.[45] These data should be used as approximations of the true incidence of cancer in the 21st century since the caveats provided earlier regarding changes in diagnostic practices, breed distributions, neutering patterns, and animal longevity preclude using them in a definitive context.

ANALYTIC EPIDEMIOLOGY

Analytic epidemiology is used to test hypotheses that various risk factors or exposures are associated with the disease (cancer). The hypotheses tested in analytic studies are often developed from data obtained in descriptive epidemiology. A cause-and-effect relationship is difficult to establish from observational studies and may be best evaluated in an experimental setting where all extraneous variables and confounders are carefully controlled. Since cancer incidence rates are generally low and the latent period is likely to be measured in years, experimental approaches to study cancer causation in dogs and cats

TABLE 4-1   Annual Crude Incidence Rates of Cancer per 100,000 Population for Humans, Dogs, and Cats, Alameda County, 1960 to 1966

Cancer site	Humans	Dogs	Cats
All sites	272.1[a, b]	381.2[c]	155.8[c]
Skin, nonmelanoma	31.8[a, e]	90.4[c]	34.7[c]
Malignant melanoma	8.3[c]	25.0[c]	ND[c]
Digestive	74.5[e]	25.2[e]	11.2[e]
Respiratory	32.9[e]	8.5[e]	5.0[e]
Connective tissue	2.4[e]	35.8[c]	17.0[c]
Mouth and pharynx	10.3[e]	20.4[c]	11.6
Breast	37.3[e]	198.8[d]	25.4[d]
Lymphoid tumors	13.3[e]	25.0[d]	48.1[d]
Bone	1.2[e]	7.9[c]	4.9[c]
Testis	2.6[e]	33.9[c]	ND

[a]Excludes squamous cell carcinoma of the skin.
[b]Data adapted from Dorn.[71]
[c]Data adapted from Dorn et al.[3]
[d]Data adapted from Dorn.[72]
[e]Data adapted from Schneider.[45]

are rarely conducted, although several notable exceptions can be cited, for example, lifetime studies of beagle dogs exposed to radionuclides.[46] However, a causal inference may be suggested from epidemiologic studies when the evidence meets several criteria including (1) the exposure precedes the development of the disease (temporality); (2) the association between the exposure and the disease can be explained by a biologically plausible mechanism and data from laboratory animal experiments supporting the association may exist (biological plausibility); (3) the association between exposure and disease is strong as measured by the relative risk or odds ratio (strength); (4) the association between the exposure and disease is relatively specific (specificity); and (5) the association has been demonstrated across a number of studies that may use different study designs and have been conducted in various populations (consistency). Although these criteria and others have been applied to assess "causality" in many situations, shortcomings in applying them rigidly have been identified.[44]

The two principal study designs that have been used in cancer epidemiology are the case-control study and the cohort study. Generally, specific forms of cancer are too rare to apply a cross-sectional design with screening techniques, unless one is examining a high-risk population. The elements of study design and analysis for case-control and cohort studies are beyond the scope of this chapter but have been described elsewhere

with applications to small animal medicine[47,48] and in standard textbooks of epidemiology.[44]

There are many examples of well-conducted case control studies that have identified new risk factors for canine and feline cancers and other disorders. These include studies of exposures to topical flea and tick products and herbicides and bladder cancer,[49,50] consumption of vegetables and decreased risk of bladder cancer,[51] exposures to secondhand smoke and canine lung and nasal cancer [52,53] and feline lymphoid cancer,[54] sunlight exposure and risk of squamous cell carcinoma in cats,[55] and exposures to flea control products and oral squamous cell carcinoma in cats.[54] The latter study led to a follow-up investigation that reported overexpression of p53 in response to secondhand smoke exposure in cats with oral squamous cell carcinoma.[56] Case-control methods have also been applied to develop new knowledge about potential risk factors for feline hyperthyroidism[57] and canine gastric dilatation-volvulus syndrome.[58] From the standpoint of veterinary oncology and medicine, these studies, if confirmed by further research, offer exciting potential for intervention to reduce the risk of cancer and other diseases in dogs and cats. Preventive approaches to canine and feline cancer have lagged behind developments in diagnosis and therapy but are critical if the burden of cancer in pet animals is to be reduced.

Cohort studies have been conducted less frequently than case-control studies in cancer epidemiology for several reasons. First, cancer is a relatively rare event, requiring large sample sizes to yield adequate numbers of cases of specific cancers to conduct analyses with adequate statistical power. Second, the effort involved in following large numbers of dogs or cats prospectively requires considerable resources. Third, although prospective studies can be conducted over a shorter time frame in animals than in humans, the estimated latent period for cancer in the dog and cat must be included in the follow-up period to evaluate the exposure-response relationship. Finally, attrition, loss to follow-up, and changes in exposure status occur over time and contribute to the difficulty of conducting prospective cohort studies. Cohort studies of cancer and other outcomes in pet animals have usually been designed to test specific hypotheses and used high-risk populations to increase efficiency. Incidence rates and relative risk can be measured directly from cohort studies using dog-years at risk to account for varying periods of observation for individual subjects.

The author's experience with cohort studies illustrates the features of the prospective design and the problems associated with this approach.[58a] Philadelphia area practitioners followed 938 dogs for up to 5 years to determine the incidence of testicular neoplasia in cryptorchid and normal animals matched for age and breed. The cohort

experienced a total of 1785 dog-years of risk. The normal (unexposed) and cryptorchid (exposed) subcohorts were matched for age and breed. At the end of 5 years, 1785 dog-years of risk had been accumulated by cohort members. Ten sertoli cell tumors and six seminomas developed in the 609 cryptorchid dogs, none in the 329 normal dogs. Nearly one third of the total cohort could not be followed to the study end point owing to death from competing causes, emigration, failure to return for examination, change in ownership, or change in exposure status (castration).

A more efficient design is the retrospective or historical cohort study. In historical cohort studies, the population is identified from previously collected information and the current status of each animal is determined (vital status, cause of death, cancer diagnosis). Cooley et al. conducted a historical cohort study of rottweiler dogs that were followed over a significant proportion of their lifetime.[59] Dog owners completed an extensive questionnaire that identified a cohort of 683 dogs that were alive in 1995. After a mean duration of approximately 8.7 years and 71,004 total months of follow-up for the entire cohort, 86 dogs were identified that had radiographic or histologic evidence of osteosarcoma. Multivariate analysis using COX proportional hazards modeling showed a strong association between gonadectomy before 1 year of age and the development of osteosarcoma that was independent of adult height or body weight. Bone sarcoma risk was dose dependent on gonadal hormone levels estimated by date of castration in male dogs.

ENVIRONMENTAL CANCER EPIDEMIOLOGY AND ANIMAL SENTINELS

An animal sentinel system is one in which data on animals exposed to contaminants in the environment are collected and analyzed to identify potential health threats.[60] Historically, health effects in animals have served as harbingers of environmentally mediated threats to human health. Deaths of fattened cattle at a London stock show occurred during a severe air pollution episode in the 1850s that caused widespread human mortality. Cats consuming fish contaminated with methylmercury developed signs of neurologic disease termed "dancing cat fever" that preceded the development of human illness at Minimata Bay, Japan. More recently, studies of malformed amphibians have raised concerns regarding human exposure to chemicals that may have endocrine disrupting properties. Animal sentinels for cancer have been identified by epidemiologic studies in dogs and cats.[61] Several noteworthy examples of environmental exposures that increase risk

of cancer in animals have been published and will be reviewed here briefly.

Mesothelioma in Dogs and Exposure to Asbestos

In 1983, Glickman et al. described a study of mesothelioma in dogs that remains a classic in its approach and significance.[62] Eighteen histopathologically confirmed cases of mesothelioma were identified from a hospital population, and owners of 16 of these dogs were interviewed as well as 32 owners of control dogs to determine the dog's medical history, lifestyle, diet, and potential exposure to asbestos. Owners of dogs with mesothelioma were more likely than owners of control dogs to have been exposed to asbestos at work or by a hobby of a household member. Higher levels of chrysotile asbestosis fibers were identified in the lungs of 3 dogs with mesothelioma and 1 dog with squamous cell carcinoma of the lung than in lung tissue from control dogs and 2 dogs with broncho-alveolar cell carcinoma. There was also a significant association with the use of flea repellants, some of which may have contained asbestos-like fibers. The findings of this study illustrate the usefulness of epidemiologic research that may identify environmental health hazards for humans who share the environment with their pets.

Lung Cancer, Tonsillar Cancer, and Urban Residence

Primary lung cancer is rare in dogs. To explore the hypothesis that these cases might be associated with exposure to air pollutants in urban dogs, Reif et al. conducted a study of respiratory tract cancers in the Philadelphia area and assigned exposure based on ambient levels of total suspended particulates.[63] The residence of dogs with lung cancer, nasal cancer, tonsillar cancer, gastrointestinal cancer, and a sample of the entire hospital population was compared. No significant association was noted in patient distribution between urban and rural zones for cancer of the lungs and bronchi or nose and paranasal sinuses. However, a significant urban association was noted for dogs with tonsillar carcinoma, 74% of which resided in the urban zone compared with 61% of the total population and 47% of a control group with gastrointestinal neoplasia.

In 1939, an association between carcinoma of the tonsil and "townkept" dogs was noted in London.[64] The prevalence of tonsillar carcinoma in London was reported to be 12/10,000,[65] and in an earlier study in Philadelphia, 9.1/10,000.[66] Conversely, Ragland noted that the prevalence in the hospital population in rural Washington was significantly lower (1/10,000),[67] and the 2 dogs from Washington were referred from large,

industrialized cities. The reported urban excess suggests that this cancer may have a role as a sentinel event that should be pursued to test more refined etiologic hypotheses. Studies suggest that dogs that develop cancer of the lung and nasal sinuses may have been exposed to environmental tobacco smoke.[52,53] From a public health perspective, smoking cessation can be recommended as a measure to reduce the incidence of human, as well as canine, respiratory tract cancers.

Bladder Cancer, Industry, and Pesticides

There are several lines of evidence that bladder cancer in dogs may be associated with exposures to environmental contaminants and that some of these agents may also be associated with human cancer risk. In both humans and dogs, most bladder cancers are transitional cell carcinomas of the urothelium.[61] Bladder cancer may be induced experimentally in dogs by administration of aromatic hydrocarbons, including the aromatic amines such as beta-naphthylamine that were used in the textile industry and associated with increased bladder cancer risk in occupationally exposed persons.[68] The induction time for chemically induced bladder cancer in dogs is shorter than that reported for humans, suggesting that dogs may be exceptionally sensitive to the effects of these chemicals.

Human bladder cancer has been consistently associated with exposures to chemicals in occupationally exposed persons. Canine bladder cancer may also be caused, in part, by exposure to chemicals in the environment. In a study by Hayes et al.,[23] the proportional morbidity ratios for bladder cancer in dogs at 13 veterinary teaching hospitals were significantly correlated with level of industrial activity in the same counties, suggesting environmental exposure to carcinogens. An analysis of mortality from bladder cancer among white men and women in the same counties showed a similar correlation with industrial activity. Therefore, the dog may provide a sentinel for detecting the effects of environmental carcinogens, relatively exempt from confounding influences such as occupation and cigarette smoking. The shorter latency period for canine bladder cancer could lead to early identification of carcinogenic hazards in the general environment.[23]

A case-control study of pet dogs was conducted to determine if exposure to environmental tobacco smoke, use of chemicals in the home, use of topical insecticides, and obesity were associated with the occurrence of bladder cancer.[49] Information was obtained by interview from owners of 59 dogs with bladder cancer and 71 age- and breed-sized matched controls with other diseases. Bladder cancer risk was unrelated to environmental tobacco smoke or to household chemical exposures. However, risk was significantly increased by the use of topical insecticides in shampoos and dips. The risk estimate for flea and tick dips was 1.6 for one to two applications per year and rose to 3.5 for more than two applications per year. This risk was enhanced among obese or overweight dogs rising to an odds ratio (OR) of 3.5 among male dogs and 27.2 among female dogs with both exposures.[49] The findings suggested that obesity may modify the risk for exposure to these chemicals by enhancing their lipophilicity in organic solvent carriers. Flea and tick dip products contain up to 96% inert ingredients including petroleum distillates and solvents such as benzene, toluene, and xylene.[61] This work focused attention on the need to consider the inert substances in pesticide formulations as potential risk factors for human bladder cancer, especially among nonoccupationally exposed, nonsmoking females.[61]

The investigators conducted several follow-up studies to evaluate the hypothesis that pesticides are associated with canine bladder cancer risk. The study design was based on case-control analyses of Scottish terrier dogs with bladder cancer and a group of adult Scottish terriers with other disorders recruited through the Scottish Terrier Club of America. Scottish terrier dogs have approximately 18 times the risk of bladder cancer compared to mixed-breed dogs;[69] other terrier breeds such as Wirehaired fox terriers and West Highland white terriers are also at increased risk, suggesting a genetic predisposition. In Scottish terriers, treatment with topical spot-on flea and tick products containing fipronil or imidacloprid within 1 year prior to diagnosis was not associated with an increased risk of bladder cancer after adjustment for host factors.[70] However, in a study of herbicide exposures, the risk of transitional cell carcinoma was significantly increased among dogs exposed to lawns or gardens treated with both herbicides or insecticides (OR 7.2) or with herbicides alone (OR 3.6) but not among dogs exposed to lawns or gardens treated with insecticides alone.[50] Phenoxy acid herbicide exposure was associated with a fourfold increase in risk for bladder cancer in Scottish terriers. Further epidemiological studies of household dogs are needed to identify specific bladder carcinogens in herbicide and insecticide products that are used on animals or in the environment and to assess the potential adverse effects in humans.[50] The Scottish terrier model provides an excellent opportunity to test gene-environment interactions in bladder cancer. Studies of gene-environment interactions will constitute the next important phase of cancer epidemiology in pet animals.

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Paraneoplastic Syndromes

Philip J. Bergman

A paraneoplastic syndrome (PNS) is a neoplasm-associated alteration in bodily structure or function that occurs distant to the tumor. PNSs are an extremely diverse group of clinical aberrations that are associated with the noninvasive actions of the tumor. In many situations, the PNS parallels the underlying malignancy, and, therefore, successful treatment of the tumor leads to disappearance of the PNS. Alternatively, recurrence of the PNS after successful treatment signals recurrence of the tumor, and the return of the PNS often significantly precedes the gross recurrence of the tumor.

PNSs are often the first sign of malignancy or may be a hallmark of a certain tumor type. Therefore, an understanding and appreciation for the types and causes of these syndromes are paramount for early cancer detection and appropriate therapy. In addition, PNS can cause greater morbidity than that associated with the actual tumor. Unfortunately, some PNSs can be so severe that their treatment may take priority over appropriate therapies instituted against the tumor, although the tumor may in fact be readily treatable (e.g., hypercalcemic lymphoma).

The causes of PNS are quite variable; however, they are usually caused by the production of small molecules, which are released into the circulation to cause effects at distant sites. Some PNSs are secondary to a germline mutation, whereas many nonendocrine PNSs have no known etiology. Though recognized more commonly in human cancer patients, PNSs do occur in veterinary medicine. Box 5-1 summarizes the most common PNS of dogs and cats with the tumors known to cause them. This chapter reviews the wide range of PNSs seen in veterinary oncology with a system-based approach, and each system section contains a brief discussion of comparative aspects to related human PNS when appropriate.

GASTROINTESTINAL MANIFESTATIONS OF CANCER

Cancer Cachexia and Anorexia

A frequent and important systemic effect of cancer in animals is profound malnutrition and body mass wasting. The weight loss and metabolic alterations observed in cancer patients despite adequate nutritional intake is termed cancer cachexia, whereas alterations observed due to poor nutritional intake is termed cancer anorexia. The clinical outcome of either cancer cachexia or anorexia is a progressive wasting (Figure 5-1). The weight loss endured by these patients is more than a simple cosmetic abnormality as human patients with cancer cachexia can have significantly reduced survival times, and many patients are unable to undergo appropriate therapy due to resulting poor clinical performance status.

Cancer cachexia occurs frequently in human oncology, with estimated incidences from 40% to 90% of hospitalized patients.[1] Importantly, cancer cachexia accounts for approximately 20% of cancer deaths.[2] The incidence of cancer cachexia in veterinary oncology patients is presently unknown; however, some authors have suggested that cancer cachexia is potentially a more significant problem in dogs than in people.[3] This author believes that an estimated incidence of cancer cachexia in dogs is realistically less than 25%; however, appropriate clinical studies remain to be done to determine the actual incidence of cancer cachexia in dogs and cats. Only 4% of dogs presenting to an oncology service were found to have cachexia in a report by Michel et al.[4] The metabolic alterations associated with this PNS usually occur before weight loss is detected in both human and veterinary cancer patients.[5-9] Unfortunately, these metabolic alterations often persist for some time after the patient

| Box 5-1 | Paraneoplastic Syndromes and Associated Tumors |

Gastrointestinal manifestations of cancer
Cancer cachexia
Multiple tumor types

Gastroduodenal ulceration
Mast cell tumor
Gastrinoma

Endocrinologic manifestations of cancer
Hypercalcemia of malignancy
Lymphoma
Anal sac apocrine gland adenocarcinoma
Multiple myeloma
Parathyroid tumors
Mammary tumors
Thymoma
Others

Hypoglycemia
Insulinoma
Hepatic tumors
Salivary tumors
Leiomyoma/leiomyosarcoma
Plasma cell tumors
Lymphoma
Others

Ectopic ACTH
Primary lung tumors

Hematologic manifestations of cancer
Hypergammaglobulinemia
Multiple myeloma
Lymphoma

Anemia
Multiple tumors

Erythrocytosis
Renal tumors (increased erythropoietin)
Lymphoma
Nasal fibrosarcoma
TVT
Hepatic tumors

Neutrophilic leukocytosis
Lymphoma
Multiple tumors

Thrombocytopenia/coagulopathies/DIC
Lymphoma
Mast cell tumor
Hemangiosarcoma
Thyroid tumors
Mammary tumors
Nasal tumors
Inflammatory carcinomas
Others

Cutaneous manifestations of cancer
Alopecia
Pancreatic carcinoma (cat)
Others

Flushing
Mast cell tumor
Pheochromocytoma
Others

Nodular dermatofibrosis
Renal cystadenoma/cystadenocarcinoma

Necrolytic migratory erythema
Glucagonoma

Cutaneous necrosis of the hind feet
Lymphoma (cat)

Pemphigus vulgaris
Lymphoma (dog and horse)

Renal manifestations of cancer
Glomerulonephritis/nephrotic syndrome
Multiple myeloma
Polycythemia vera
Lymphocytic leukemia
Others

Neurologic manifestations of cancer
Myasthenia gravis
Thymoma
Osteosarcoma
Biliary carcinoma
Others

Peripheral neuropathy
Insulinoma
Others

Miscellaneous manifestations of cancer
Hypertrophic osteopathy
Primary lung tumor
Urinary bladder rhabdomyosarcoma
Esophageal tumors
Metastatic tumors
Others

Fever
Multiple tumors

Figure 5-1

Dog with lymphoma and secondary severe cachexia. Cancer cachexia can be a common paraneoplastic syndrome in dogs and cats. The weight loss noted in cases of paraneoplastic cancer cachexia occurs despite adequate nutritional intake. The metabolic alterations associated with cancer cachexia usually occur before clinical signs of the inciting malignancy appear and unfortunately continue after the patient is successfully treated for the tumor.

is rendered tumor free making reversal of weight loss difficult.[6,8]

In the clinical evaluation of a veterinary patient for the possibility of cancer cachexia/anorexia, a detailed history and physical exam are crucial. The prognostic importance of the presence of cancer cachexia in human cancer patients cannot be overstated, as many studies show that this PNS is the only or one of very few independent multivariate negative prognostic factors for a variety of malignancies.[1] Cancer cachexia in dogs and cats is an important PNS and potentially important prognostic factor that should be closely evaluated in subsequent veterinary cancer studies.

Protein-Losing Enteropathy

Protein-losing enteropathy (PLE) is a syndrome whereby excessive serum proteins are lost into the gastrointestinal (GI) tract leading to hypoproteinemia.[10] The hypoproteinemia seen in cancer patients can be due to impaired synthesis or increased loss into the GI tract or urine (see Renal Manifestations of Cancer, later in this chapter). Once the loss of proteins becomes greater than the body's ability to synthesize them, serum protein levels begin to decrease. The half-life of many serum proteins is extremely long, and therefore those patients with hypoproteinemia due to PLE or some other cancer-related protein loss typically have a longstanding protein loss.[1,10] PLE is thought to result from an increase in mucosal serum protein permeability due to mucosal erosion, ulceration, or lymphatic obstruction.

The diagnosis of PLE is made by noting hypoproteinemia on serum chemistry evaluation with subsequent exclusion of severe malnutrition and liver disease. Confirmation of the diagnosis is made in people by alpha-1-antitrypsin detection in stool;[11] however, this methodology has not been extensively evaluated in veterinary medicine. In addition, nuclear scintigraphy (**[111]indium-labeled transferrin**) appears to be a reliable methodology for the diagnosis of PLE.[12] The incidence of PLE as a PNS is unknown in veterinary medicine but is likely to be rare. The treatment for PLE consists of treating the primary malignancy; however, those patients with a lymphangiectasia-related PLE may also be treated with medium-chain triglycerides that do not undergo transport by intestinal lymphatics.

Gastroduodenal Ulceration

The most common cause of PNS-associated gastroduodenal ulceration is mast cell tumor (MCT). The excess histamine seen in MCTs stimulates gastric H_2 receptors leading to increased gastric acid secretion. Clinical end points of mucosal damage or ulceration with gastric vessel thrombosis occur in association with gastric hyperacidity. Plasma histamine concentrations are elevated in approximately 75% of dogs with macroscopic MCT, whereas only a minority of dogs with MCT have gastrointestinal signs.[13,14] Abnormally elevated plasma histamine concentrations have also been found to be a negative prognostic factor in dogs with MCT.[14] Symptomatic therapies such as proton pump inhibitors, H_2 blockers, misoprotol, sucralfate, and rehydration may be helpful in combating PNS-associated gastroduodenal ulceration. Mast cell tumors are covered in greater detail in Chapter 19.

An additional cause of PNS-associated gastroduodenal ulceration is gastrinoma (gastrin-secreting nonislet cell pancreatic tumor). Though these tumors are rare, they have been reported in both dogs and cats.[15–19] Gastrinomas can be associated with vomiting, lethargy, anorexia, blood loss, and abdominal pain. Many of these features are also seen in humans with gastrinoma-related Zollinger-Ellison syndrome. Gastrinomas are covered in greater detail in Chapter 24.

ENDOCRINOLOGIC MANIFESTATIONS OF CANCER

Hypercalcemia

The most common cause of hypercalcemia in the dog is cancer. A variety of tumors have been associated with hypercalcemia of malignancy (HM). Neoplasia is diagnosed in approximately two thirds of dogs with hypercalcemia[20,21] and approximately one third of cats.[22]

Lymphoma is the most common cause of HM, and the most common anatomic site for dogs with lymphoma-associated HM is the cranial mediastinum. Other tumors associated with HM in dogs and cats include anal sac apocrine gland adenocarcinoma, thyroid carcinoma, multiple myeloma, bone tumors, thymoma, squamous cell carcinoma, mammary gland carcinoma/adenocarcinoma, melanoma, primary lung tumors, chronic lymphocytic leukemia, and parathyroid gland tumors.[23–29] The incidence of HM in veterinary oncology patients in unknown, however, it is seen in 10% to 35% of dogs with lymphoma, >25% of dogs with anal sac apocrine gland adenocarcinoma, and approximately 20% of dogs with myeloma.[30–31] HM is seen in 5% to 30% of human cancer patients.[1]

The causes of HM are varied and include ectopic production of parathormone (PTH) or PTH-related peptide (PTH-rp) by the tumor, extensive lytic bone metastases, primary hyperparathyroidism, tumor-associated prostaglandins ($PGE_{1/2}$), interleukin-1 Beta (IL-1, previously known as osteoclast activating factor, OAF), transforming growth factor-β (TGF-β), and receptor activator of nuclear factor Kappa Beta ligand (RANKL).[1,27,32–36] Interestingly, TGF-β1 regulates mRNA stability of PTH-rp.[37] The HM seen in lymphoma and anal sac apocrine gland adenocarcinoma is commonly caused by tumor-associated PTH-rp.[38,39] PTH-rp is a 16kDa protein with significant sequence identity to PTH. In addition to HM, other differentials of hypercalcemia such as lab "error" (lipemia and hemolysis), acute renal failure, hypervitaminosis D, hypoadrenocorticism, granulomatous disease, and others should be considered.

In addition to ensuring the hypercalcemia is not due to lipemia or hemolysis, it is important to interpret the total calcium value in relation to the level of serum albumin. Two commonly utilized correction formulas that account for the level of serum albumin follow:

Adjusted calcium (mg/dL) = [Calcium (mg/dL)
$$- \text{albumin (g/dL)}] + 3.5$$
or
Adjusted calcium (mg/dL) = Calcium (mg/dL)
$$- [\text{Total serum protein (g/dL)} \times 0.4] + 3.3$$

Similarly, an increase in the free ionized fraction of calcium can occur with acidosis. Acidotic HM patients may have an increase in clinical signs of hypercalcemia when compared to nonacidotic HM patients.

The primary clinical manifestations of HM are due to renal function impairment. Severe HM (calcium >18 mg/dL) should be considered a *medical emergency*. An inability to concentrate urine is noted first due to decreased responsiveness to ADH at the distal tubule; the calcium then decreases renal blood flow and glomerular filtration rate (GFR) due to severe vasoconstriction.

Calcium salt deposition in the renal parenchyma further compounds the pre-renal and renal azotemia. The urinary epithelium may then undergo degeneration and, in severe cases, necrosis. The situation clinically worsens as the patient becomes severely polyuric and polydipsic, begins vomiting, and then undergoes continual and progressive dehydration. In addition to its effects on the renal system, in severe cases of HM one may see constipation, hypertension, twitching, weakness, shaking, depression, vomiting, bradycardia, stupor, and possibly coma or death.

The diagnosis of the etiology of HM can be difficult in some cases. Other laboratory findings commonly seen in HM cases include azotemia with hypo- to normophosphatemia. Advanced HM cases with severe renal destruction may have hyperphosphatemia, even though it is an uncommon feature of this PNS. When true HM has been diagnosed, appropriate steps for identification of the cause are immediately necessary. When azotemia is present in HM cases, this represents a true *medical emergency* for delineation of the cause of the HM and for appropriate therapy. The diagnostic steps for HM should include those undertaken for the staging of lymphoma (as outlined in Chapter 31) in addition to a rectal palpation/examination for anal sac apocrine gland adenocarcinoma. If these diagnostics do not confirm the specific cause of the HM, then the aforementioned hypercalcemia differentials should be considered and appropriately pursued. Dogs and cats with HM will typically have low PTH (unless a primary parathyroid tumor is responsible) and high PTH-rp concentrations; however, the cause of the HM can usually be delineated before the return of PTH/PTH-rp assay results. Similarly, the astute clinician will remember that there are causes of HM other than lymphoma or anal sac apocrine gland adenocarcinoma, and therefore a thorough physical examination and staging is tantamount to successful delineation of the causes in HM cases.

Since HM is a potential medical emergency, the primary goal in cases of HM is the elucidation of the underlying cause and subsequent institution of the appropriate specific therapy. Though often necessary when searching for the underlying cause of the HM, symptomatic therapy must be judiciously utilized. The premature administration of symptomatic therapy such as the use of corticosteroids prior to the confirmation of the cause of the HM can significantly affect and delay diagnosis. If lymphoma or myleoma is the underlying cause of the HM, the use of corticosteroids may interfere with the ability to confirm the diagnosis, necessitating either additional diagnostics or waiting to determine if the lymphoma or myeloma reappears after glucocorticoid administration is discontinued. In addition to the implications of diagnostic interference, glucocorticoids may induce resistance to other chemotherapy agents with a decrease in the ability to induce a complete

remission as well as a decrease in the length of survival (see Chapter 31 for further discussion).[40] Therefore, the use of corticosteroids in cases of undiagnosed hypercalcemia is strongly discouraged.

Symptomatic therapies that promote external loss of calcium, increase renal excretion of calcium, and inhibit bone reabsorption may be utilized in HM patients. The severity of clinical signs and associated hypercalcemia determine the preferred therapy. The use of 0.9% NaCl intravenously (IV) is commonly utilized to ameliorate the aforementioned dehydration with expansion of the extracellular fluid volume. In addition, the IV 0.9% NaCl will increase GFR, increase calciuresis and natruresis, and decrease calcium reabsorption by the kidneys. *Once fully rehydrated*, the loop diuretic furosemide with continued normosaline diuresis can be utilized to potently inhibit calcium reabsorption in the ascending loop of Henle. If the specific cause of the HM is delineated, corticosteroids can be extremely effective in the adjunct treatment of HM by its inhibition of prostaglandin E (PGE), OAF, vitamin D, and intestinal calcium absorption. Corticosteroids can also be cytotoxic to lymphoma and myeloma cells. The most common therapies utilized in the treatment of HM are outlined in Box 5-2. In rare cases

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Box **5-2**	**Treatment for Hypercalcemia of Malignancy**

Elimination of the inciting tumor is the primary goal for all categories of hypercalcemia!

Mild hypercalcemia and minimal clinical signs
Rehydration with normosaline (0.9% NaCl) if dehydrated. If the patient has no clinical signs and is eating and drinking and well hydrated, treating the underlying cause of the HM as an outpatient is acceptable.

Moderate hypercalcemia and clinical signs
Rehydration with normosaline (0.9% NaCl)
Continue normosaline diuresis (urine output > 2ml/kg/hr)
Furosemide (1–4 mg/kg every 8–24 hours IV or PO)
Note: Only use after patient is fully rehydrated.
Prednisone (1 mg/kg daily to BID PO)
Note: Only use after diagnosis obtained (see text).

Severe hypercalcemia and severe clinical signs
Oncologic emergency!
See moderate hypercalcemia treatments
For refractory cases:
　Salmon calcitonin (4–10 MRC units/kg SQ daily)
　Biphosphonates
　　Pamidronate (1–1.5 mg/kg IV, every 2–4weeks)
　　Zoledronate (0.25 mg/kg IV, every 4–5 weeks)

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that are unresponsive to symptomatic therapies and treatment of the underlying cause, calcitonin or parenteral bisphosphonates may be utilized.[41] Bisphosphonates have become standard therapy for human nonhumoral HM due to their potent inhibition of bone resorption without affecting tubular calcium reabsorption.[42] The use of bisphosphonates in dogs and cats appears to be a promising new treatment for hypercalcemia.[43,44] In the future, likely treatment options for HM may include osteoprotegerins, more potent bisphosphonates, anti-PTH-rp antibodies, noncalcemic calcitriol analogues, distal tubule calcium reabsorption inhibitors, and new bone resorption inhibitors.[42,45]

Hypoglycemia

The most common causes of hypoglycemia (less than 65 to 70 mg/dL serum glucose) in the dog is insulinoma (beta-islet cell tumor; see Chapter 24).[46] Interestingly, the most common cause of PNS-associated hypoglycemia in people is mesothelioma;[1] however, this has not been reported to be a cause of PNS-associated hypoglycemia in veterinary oncology to date. Nonislet cell tumors can also serve as sources of ectopic hormone production with resultant hypoglycemia in dogs and humans. Nonislet cell tumors with PNS hypoglycemia have been most commonly associated seen in hepatocellular carcinomas; however, lymphoma, hemangiosarcoma, leiomyosarcoma, oral melanoma, hepatoma, plasma cell tumor, multiple myeloma, smooth muscle tumors (Figure 5-2; leiomyoma and leiomyosarcoma), and salivary gland tumors have also been reported.[47,48] The hypoglycemia

Figure 5-2

A duodenal leiomyoma in an 8-year-old Husky with seizures and a serum glucose of 21 mg/dL. Serum glucose returned to normal within 48 hours of surgical extirpation of the tumor.

of extrapancreatic tumors has interestingly been associated with low insulin levels, whereas pancreatic beta-islet cell tumors (insulinomas) induce hypoglycemia by excessive circulating insulin levels. Nonislet cell tumors may induce hypoglycemia by increased tumor utilization of glucose, decreased hepatic glycogenolysis or gluconeogenesis, or the secretion of insulin or insulin-like growth factor I and II (IGFI and IGFII). Additional mechanisms include upregulation of insulin receptors, increased insulin binding by M-proteins in myeloma, and increased production of somatomedins.[1,49] The differential diagnosis of hypoglycemia includes insulinoma, nonislet cell tumor hyperinsulinism, nonislet cell tumor, hypoadrenocorticism, starvation, sepsis, liver dysfunction, and laboratory error (lack of timely serum separation).

Clinical signs of neuroglycopenia due to hypoglycemia are common when the serum glucose drops below 45 to 50 mg/dL. Such clinical signs include weakness, disorientation, seizures, and coma, which are due to the strict need for continuously available glucose in peripheral and central nervous tissue.[50] The threshold for the development of clinical signs due to hypoglycemia can be variable and are often related to acuteness of onset. Dogs with longstanding hypoglycemia may not have any clinical signs of hypoglycemia at a serum glucose of 40 mg/dL, whereas dogs with acute hypoglycemia for the first time may have seizures at a serum glucose of 45 to 50 mg/dL. To compensate for the hypoglycemia, counterregulatory hormones such as catecholamines, glucocorticoids, glucagon, and growth hormone are released to promote glycogenolysis and gluconeogenesis.

The diagnosis of insulinoma or other extrapancreatic insulin-producing tumors is made by identifying concomitant hypoglycemia with elevated serum insulin levels. Though a significant proportion of cases will be diagnosed by this concurrent testing, occasional cases may necessitate a 48- to 96-hour fast with periodic concurrent sampling of blood glucose and insulin. Due to the possibility of life-threatening hypoglycemia with such a lengthy fast, 24-hour care is recommended during this period. The amended insulin-glucose ratio (AIGR) is a controversial but useful diagnostic ratio for aiding in the diagnosis of an insulinoma. The formula for AIGR follows:

$$AIGR = [Serum\ insulin\ microU/ml) \times 100] / [Serum\ glucose\ (mg/dl) - 30]$$

An AIGR of greater than 30 suggests a diagnosis consistent with but not diagnostic for insulinoma or, rarely, a nonislet insulin producing tumor.[50] An AIGR < 30 does not rule out insulinoma or nonislet insulin-producing tumor, and it suggests that additional testing is required if all signs are consistent with tumor-associated hyperinsulinemia. Amelioration of neuroglycopenic signs upon treatment with glucose-containing solutions is additional

supportive evidence for the diagnosis of insulinoma or non-islet insulin producing tumor. Tumors associated with extrapancreatic hypoglycemia are often large and therefore radiographs/ultrasound of the abdomen or thorax may be helpful. If a mass is found, biopsy with histopathologic confirmation is recommended; however, if no mass is found, some cases require exploratory laparotomy, especially since most insulinomas are too small for routine imaging preoperatively. Provocative testing via glucagon or glucose tolerance testing may be useful in cases when the diagnosis is uncertain.

The treatment of choice for tumors that produce PNS hypoglycemia is surgical extirpation. Since insulinomas are the most common cause of paraneoplastic hypoglycemia, partial pancreatectomy is indicated with careful evaluation of local lymph nodes and the liver.[51] Uncommon complications of partial pancreatectomy are transient iatrogenic diabetes mellitus and pancreatitis. The use of medical therapies and alterations in feeding regimens are many times necessary prior to, during, and after surgical extirpation of these tumors. Prednisone (1 to 2 mg/kg divided BID PO) is one of the most widely utilized adjunct medical therapies for PNS hypoglycemia due to its ability to increase serum glucose.[52] An additional medical agent that may be useful in the adjunct treatment of PNS hypoglycemia is diazoxide (10 to 60 mg/kg divided BID PO).[52] Diazoxide can be difficult to obtain and may be cost prohibitive for some clients; however, it can be effective in increasing serum glucose levels by enhancing epinephrine-induced glycogenolysis and inhibiting insulin release and uptake by cells.[52,53] Propranolol is a beta-adrenergic blocking agent (10 to 30 mg/kg TID PO) that may increase serum glucose concentrations;[54,55] however, this author has not found propranolol to be useful in the medical management of paraneoplastic hypoglycemia. Small frequent feedings and glucose-containing solutions given PO or IV can be extremely simple and useful techniques for the medical management of these tumors. Other therapies include hydrochlorothiazide (2 to 4 mg PO BID; may potentiate effects of diazoxide), glucagon (0.03 mg/kg IV), and somatostatin (Octreotide acetate at 5 to 20 :g PO or SQ BID-TID). Streptozotocin (10 to 20 mg/kg IV) is an antineoplastic agent with some specificity for the pancreatic islet cell that may prove to be useful in the adjunct treatment of these tumors. This agent has historically not been recommended due to nephrotoxicity,[56,57] but evidence suggests this may be abrogated by concurrent saline diuresis.[58] See Chapter 24 for additional diagnostic and prognostic information regarding insulinoma.

Syndrome of Inappropriate Secretion of Antidiuretic Hormone (SIADH)

SIADH is a PNS widely recognized in lung, head, and neck, and other tumors in humans;[1] however, it continues

to be essentially unrecognized in veterinary oncology. In addition to PNS-associated SIADH, chemotherapy agents and other drugs (vincristine, cyclophosphamide, cisplatin, thiazides, morphine, and chlorpropamide), pulmonary or CNS infections, and a variety of other conditions can cause SIADH.[59,60] The initial finding in SIADH patients is hyponatremia. In addition to hyponatremia, serum hypo-osmolarity, hypernatruresis, urine hyperosmolarity, and euvolemia with normal renal, thyroid, and adrenal function are noted.[1,59] Though most human SIADH patients are asymptomatic, clinical signs can develop due to hyponatremia, which result in CNS signs such as fatigue, anorexia, confusion, and potentially seizures. The treatment of choice for PNS-associated SIADH is removal of the underlying cause. In addition, water restriction, demeclocycline (ADH antagonist), and hypertonic sodium chloride may be useful in SIADH cases.[61]

Ectopic Adrenocorticotropic Hormone (ACTH) Syndrome

The ectopic production of ACTH or ACTH-like substances was the second PNS reported in humans.[1] This PNS is associated with small cell lung tumors, pancreatic tumors, and a wide variety of other human tumors.[1,59] In the dog, this PNS is only reported to occur in primary lung tumors.[62] The predominant active molecules in this PNS are ACTH, ACTH precursors, endorphins, enkephalins, and melanocyte-stimulating hormone (MSH).[1,59] All result in excessive production of corticosteroids from the adrenal glands resulting in clinical signs similar to those seen in hyperadrenocorticism (Cushing's disease). Invariably, tumors that cause this PNS have cortisol levels that do not suppress with dexamethasone. The diagnosis of this PNS is made by the concomitant presentation of Cushing's-like signs with an abnormal dexamethasone suppression test and a localizable tumor. The treatment of choice is removal of the underlying cause by surgical extirpation of the tumor. When necessary, medical therapy centers on inhibiting cortisol production by mitotane or ketoconazole. To date, the use of Anipryl (selegeline) in the management of this PNS in dogs has not been evaluated.

Hypocalcemia/Hyperglycemia

Paraneoplastic hypocalcemia and hyperglycemia are extremely rare. Tumors associated with lytic bone metastases and those that secrete calcitonin (medullary carcinoma of the thyroid) are the most common causes of human PNS hypocalcemia.[1] In humans, a variety of nonthyroid cancers such as breast, GI, carcinoids, and lung cancer have been reported to secrete calcitonin.[63] Most cases of PNS hypocalcemia are asymptomatic; however, human patients may have neuromuscular irritability or tetany. A gingival vascular hamartoma in a 4-month-old kitten has been reported to be associated with PNS hyperglycemia, and the hyperglycemia resolved 24 hours after removal of the tumor.[64] Treatment of choice for either PNS is eradication of the primary tumor, calcium infusion in severe hypocalcemia cases, and diabetes-like support for severe hyperglycemia cases.

HEMATOLOGIC MANIFESTATIONS OF CANCER

Hypergammaglobulinemia

Monoclonal gammopathies can be common in animals and people with cancer, and they are termed M-component disorders.[1,65–67] The hypergammaglobulinemia seen as a PNS is due to the excessive production of proteins from a monoclonal line of immunoglobulin-producing plasma cells or lymphocytes. When the production of these immunoglobulins (Igs), partial Igs, heavy chains, or light chains becomes extreme, clinical signs of hyperviscosity (ataxia, depression, dementia, cardiac disease or failure, seizures, and coma), tissue hypoxia, bleeding (poor platelet aggregation, platelet coating with Igs and release of platelet factor III), or ocular disorders (papilledema, retinal hemorrhage, detachment, etc.) may occur. These proteins may be identified by performing a protein electrophoresis on the serum or urine.[67] Similarly, light chain production may be detected in the urine as Bence-Jones proteins. In addition to the hypergammaglobulinemia PNS seen in plasma cell tumors (multiple myeloma and extramedullary plasmacytoma), lymphomas, lymphocytic leukemias, and primary macroglobulinemia can also cause this PNS.[66,67] Further discussion of plasma cell tumors can be found in Chapter 31, Section D.

Anemia

Anemia is one of the most common PNS seen in veterinary and human oncology. Approximately 20% to 25% of human cancer patients have PNS anemia and though the exact incidence of PNS anemia in veterinary oncology is unknown, it is thought to be a significant problem.[68] Though there are numerous possible causes for PNS anemia in veterinary oncology patients, the vast majority are due to either anemia of chronic disease (ACD), immune-mediated hemolytic anemia (IMHA), blood loss anemia, or microangiopathic hemolytic anemia (MAHA).

ACD is extremely common in veterinary and human oncology patients with disseminated or metastatic tumors. This anemia is due to disordered iron storage and metabolism, shortened RBC life span, and occasionally due to decreased bone marrow response.[68] The anemia seen in ACD is normocytic/normochromic, and

evaluation of the bone marrow does not suggest significant cellularity problems. Treatment of choice is removal of the tumor.

IMHA can be triggered by tumors in animals and people. Immune mechanisms then result in the premature destruction of RBCs.[69] The diagnosis of PNS IMHA is by a Coomb's slide agglutination test, and many patients will have concurrent spherocytosis and a regenerative anemia. The treatment of choice is removal of the tumor; however, if this is not immediately possible, the use of immunosuppressive dosages of prednisone (1 to 2 mg/kg daily to BID PO) may be indicated if a diagnosis has been established. Similar to non-PNS IMHA, the use of additional agents such as azathioprine (1 to 2 mg/kg daily for 4 to 7 days then 0.5 to 1 mg/kg every 48 hours PO), cyclosporine, cyclophosphamide, and others may be necessary for complicated IMHA cases.[69–72]

Blood loss anemia can be a sequel to many types of cancer. Due to decreased hemoglobin content, the RBCs in blood loss anemia are microcytic/hypochromic. In addition, decreased serum iron, increased total iron binding capacity, and poikilocytosis may also be noted.[73,74] Thrombocytosis may be seen secondary to chronic iron deficiency. The blood loss may be readily apparent in some patients (e.g., bleeding splenic tumor or bleeding superficial tumor), whereas others may not have such a readily identifiable source for the blood loss (e.g., GI tumors). The treatment of choice is removal of the tumor; however, severe anemias must be treated with blood transfusions. The use of oral ferrous sulfate (10 to 40 mg daily PO) may be a useful adjunct therapy.

MAHA is a secondary phenomenon to hemolysis and is typically due to fibrin deposition or endothelial damage.[68] The most common causes of MAHA are PNS disseminated intravascular coagulation (DIC) and RBC shearing due to hemangiosarcoma.[68,71] Schistocytosis and hemolysis are common indicators of ongoing MAHA. Almost any tumor can cause DIC and subsequent MAHA; however, hemangiosarcoma is most commonly implicated.[75] The treatment of choice is removal of the tumor; however, additional ancillary treatments such as aggressive supportive therapy, transfusions, plasma, and others may be useful.

Chemotherapy-induced anemia can be common in people due to the higher doses and greater frequencies of administration used in physician-based oncology.[1] This is rarely seen in veterinary oncology patients,[68,73,74] and the degree of anemia in veterinary cancer patients undergoing chemotherapy is generally mild with typical PCVs stabilizing in the 28% to 32% range for dogs and the 24% to 28% range for cats. This anemia rarely necessitates therapy and resolves upon discontinuation of chemotherapy.

An uncommon cause of PNS-associated anemia is myelophthisis (bone marrow invasion/crowding out),

and it is most commonly caused by leukemias.[1] Also uncommon is bone marrow hypoplasia due to hyperestrogenism. Sertoli cell tumors in the male dog and granulosa cell tumors of the female dog are associated with hyperestrogenism, and anemia is a common presenting feature.[76–78]

Erythrocytosis

Erythrocytosis is an uncommon PNS. Tumors associated with PNS erythrocytosis include renal tumors (primary and secondary); lymphoma, lung, or liver tumors; cecal leiomyosarcoma; nasal fibrosarcoma; and transmissible venereal tumor.[79–83] The erythrocytosis seen in cancer patients can be due to overproduction of erythropoietin, indirect excess erythropoietin from renal hypoxia, or increased production of hypoxia-inducible factors such as HIF-1.[84] Some tumor suppressor genes (TSG) are important proteosomal regulators of HIF-1, which may explain some of the vascular diseases seen in humans with certain TSG mutations.[85] Other differentials for polycythemia include arteriovenous shunts, severe dehydration, hyperadrenocorticism, polycythemia vera (primary polycythemia), and a variety of cardiac or pulmonary diseases.

It is important for the clinician to differentiate the various causes of erythrocytosis. Polycythemia vera is a myeloproliferative disorder resulting in clonal proliferation of RBCs with splenomegaly and possibly pancytosis.[1] Secondary polycythemia will have decreased arterial oxygen saturation. PNS erythrocytosis is best treated by removal of the erythropoietin-producing tumor; however, phlebotomy can be a useful temporary adjunct therapy. Unfortunately, the volumes typically needed for a therapeutic phlebotomy in PNS erythrocytosis cases necessitate administration of fluids and potentially readministration of plasma. The use of hydroxyurea (40 to 50 mg/kg divided BID PO)[82] as a chemotherapeutic agent for polycythemia vera has been previously recommended; however, this author has noted limited benefit with the use of this agent.

Neutrophilic Leukocytosis

Increases in circulating neutrophils have occasionally been associated with a variety of tumors in humans and dogs. Unfortunately, a leukemoid reaction of this nature can be difficult to distinguish from a true leukemia without significant diagnostic assessment. Neutrophilic leukocytosis has been reported in dogs with lymphoma, renal carcinoma, primary lung tumor, rectal polyp, and metastatic fibrosarcoma.[86–89] The exact mechanism of PNS leukemoid reactions is unknown; however, the production of a colony-stimulating factor such as granulocyte-macrophage colony stimulating factor (GM-CSF) or G-CSF are considered likely. Reports have documented

tumor-produced G-CSF and GM-CSF in dogs with primary lung tumor and renal transitional cell carcinoma. Tumor-produced G-CSF has been documented in a cat with dermal adenocarcinoma and suspected in a cat with pulmonary squamous cell carcinoma.[90–92] This PNS is generally of minimal clinical significance, and normalization is possible following removal of the inciting tumor.

Thrombocytopenia

Thrombocytopenia in human and veterinary cancer patients is typically secondary to chemotherapy administration; however, the incidence of thrombocytopenia in tumor-bearing dogs prior to chemotherapy administration has been reported to be as high as 36%.[71] Thrombocytopenia has been reported in 58% of dogs with lymphoproliferative tumors and 20% of cats with various tumors, lymphoma in particular.[93,94] Dogs with Sertoli cell tumors (or occasionally seminomas) that excessively produce estrogen are also prone to thrombocytopenia.[76,78]

Mechanisms for PNS thrombocytopenia include increased platelet destruction, platelet sequestration/consumption, or decreased platelet production. The most common tumors associated with PNS thrombocytopenia are vascular tumors of the spleen and tumors infiltrating the marrow such as lymphoma or leukemias.[95] Immune-mediated thrombocytopenia (ITP) is an additional cause of thrombocytopenia.[96] The treatment of PNS-associated thrombocytopenia is removal of the inciting tumor; however, adjunctive therapies such as intravenous fluids, plasma, and heparin may be of benefit. For those cases secondary to ITP, the use of immunosuppressive drugs such as corticosteroids (>2 mg/kg PO daily) and azathioprine (2 mg/kg PO daily then 0.5 to 1 mg/kg PO every other day) may be necessary.

Coagulopathies and Disseminated Intravascular Coagulation (DIC)

Alterations in hemostasis are common in human and veterinary cancer patients. PNS coagulopathies are most commonly associated with tumors that cause thrombocytopenia, thrombocytosis, DIC, platelet dysfunction, changes in platelet aggregation, or hyperheparinemia (due to mast cell tumor).[97,98] Trousseau's syndrome is a carcinoma-associated human coagulopathy reported in the late 1800s that appears to be related to excess production of hypoxia factors leading to angiogenesis and a procoagulant state.[99] PNS-associated DIC has been reported to cause consumptive thrombocytopenia in almost 40% of DIC cases,[71] suggesting it may be an important clinical syndrome. The diagnosis of DIC is made when the patient has thrombocytopenia, prolongation of activated partial thromboplastin time (APTT),

elevated fibrin degradation products (FDP), and hypofibrinogenemia.[71,100,101] A reduction in serum antithrombin III (AT III) levels appears to be one of the most reliable and prognostic measurements for the presence of DIC.[93,102,103]

The incidence of DIC in dogs with malignant tumors is approximately 10%.[104] A variety of tumors have been associated with DIC; however, hemangiosarcoma is most commonly implicated in dogs. Inflammatory mammary gland tumors, thyroid carcinomas, primary lung tumors, and intra-abdominal carcinomas have also been associated with DIC.[102,104–106] The presence and severity of DIC, while a significant negative prognostic factor in human cancer patients, has not been thoroughly evaluated for predictive value in veterinary patients. Though not specifically related to DIC, the hyperheparinemia associated with mast cell tumors can be associated with prolonged bleeding times and poor hemostasis after biopsy or surgery.[13]

Miscellaneous

Thrombocytosis is an extremely common PNS in humans with lymphomas and leukemias; however, it is rarely noted in veterinary oncology. Myeloproliferative disorders appear to be the most common cause of PNS thrombocytosis in dogs and cats.[107–109] Important differentials include causes of primary thrombocytosis such as inflammatory processes, some hemolytic anemias, posthemorrhage, iron deficiency, and postsplenectomy.

PNS eosinophilia is rarely reported in veterinary medicine. Dogs with mammary tumors or fibrosarcoma and cats with a variety of tumors (lymphoma, sarcomas, mast cell tumor, and bladder tumors) have been reported to have significant tumor-associated eosinophilia.[110–112] In addition, eosinophilic effusions have been documented in dogs and cats with cancer and non-neoplastic conditions.[113] The cause of eosinophilia is poorly understood; however, the production of GM-CSF, various interleukins (e.g., IL-5, 13, and 17), and eotaxins are likely candidates. PNS-eosinophilia should be distinguished from eosinophilic leukemia and hypereosinophilia syndrome, which are covered in greater detail in Chapter 31. The treatment for PNS eosinophilia is removal of the inciting tumor; however, PNS-associated eosinophilia is typically of little clinical significance.

Platelets have been suspected of playing a role in cancer progression and metastasis that has mainly been attributed to platelet aggregation-mediated augmentation of tumor cell extravasation, survival, and angiogenesis. Platelet aggregation was investigated in a study of 59 dogs with cancer.[98] When compared to control dogs, the platelets of dogs with cancer exhibited significantly higher maximum aggregation, higher ATP secretion, and shorter delays in the aggregation response. In addition

to aiding the metastatic process, platelet hyper-aggregation may also lead to thromboembolism.[114]

CUTANEOUS MANIFESTATIONS OF CANCER

A variety of cutaneous syndromes are associated with malignancies in humans,[115] whereas relatively few paraneoplastic cutaneous syndromes are noted as paraneoplastic in veterinary medicine.[116] Sweet syndrome is a human PNS associated with hematologic and solid tumors that causes acute febrile neutrophilic dermatosis.[117] As with any PNS, cutaneous-associated PNS lesions may precede, coexist, or follow the diagnosis of the underlying tumor.

Alopecia

Pancreatic carcinoma with metastasis to the liver has been reported in cats as the cause of a progressive, nonscarring PNS alopecia.[118–121] The alopecia is acute, bilaterally symmetrical (ventrum and limbs), and ventrally glistening. The hair easily epilates from nonalopecic areas and histologically exhibits severe follicular and adnexal atrophy with absence of stratum corneum in many areas including the footpads. Clinical signs include anorexia, weight loss, lethargy, and difficulty walking or standing, likely due to the aforementioned footpad histologic changes. In two reports, a similar presentation was noted in cats with bile duct carcinoma.[120,122] Differentials for this presentation include symmetrical alopecia, hyperadrenocorticism, self-induced alopecia, and telogen defluxion. The cause of this PNS is presently unknown. The prognosis appears extremely poor due to the difficulties of antemortem diagnosis; however, staging with full physical examination, blood work/ urinalysis, radiography, and abdominal ultrasound are warranted if the aforementioned differentials are ruled out.

Cutaneous Flushing

Cutaneous flushing (CF) occurs when the skin episodically turns various shades of red due to changes in blood vessel vasodilation. Intermittent or paroxysmal CF can be associated with pheochromocytoma (see Chapter 24).[1,123,124] CF has been reported in a dog with primary lung tumor and concomitant intrathoracic mast cell tumor.[125] Dogs that undergo mast cell tumor degranulation may also have CF; however, the severity is typically less than that seen in dogs with pheochromocytoma. Important non-neoplastic differentials for CF include drug reactions, demodicosis, and systemic lupus erythematosus. In addition to pheochromocytoma, humans may experience CF due to Zollinger-Ellison syndrome, carcinoids, leukemias, renal cell carcinoma, and many other conditions.[1,126]

Nodular Dermatofibrosis

Nodular dermatofibrosis (ND) is a well-recognized PNS characterized by multiple slowly growing cutaneous nodules in dogs with bilateral renal cysts or cystadeno-carcinomas (Figure 5-3).[127–130] The nodules are composed of extremely dense but well-differentiated collagen tissue (collagenous nevi) and are found predominately on the limbs, though the head and trunk may be affected in advanced cases. ND appears to be inherited in an autosomal dominant fashion and is most commonly seen in middle-aged German shepherd dogs.[127–129,131] The ND-associated mutation has been mapped to exon 7 of the Birt-Hogg-Dube (BHD) locus on canine chromosome 5, the same locus for a phenotypically similar human disease.[132] Interestingly, intact females with ND are also at increased risk for the development of uterine

Figure 5-3

Diffuse nodular dermatofibrosis (ND) in a German shepherd dog. The nodules are composed of extremely dense but well-differentiated collagen tissue (collagenous nevi) and are found predominately on the limbs, though the head and trunk may be affected in advanced cases.

leiomyomas, and the BHD mutation appears to be homozygous lethal.[129,132,133] The pathogenesis of ND is unknown at present; however, the BHD mutation in humans leads to a novel protein called folliculin, which is presently under further study.[134] There is presently no effective therapy for the underlying tumor; however, palliative therapy via surgical removal of nodules may be utilized in cases where the nodules are ulcerated, cosmetically displeasing, or are interfering with function.

Necrolytic Migratory Erythema/ Superficial Necrolytic Dermatitis

Superficial necrolytic dermatitis (SND) is an extremely rare PNS in humans and dogs characterized by circinate and gyrate areas of erosive blistering and erythema due to glucagonomas (glucagon-secreting pancreatic alpha-cell tumor).[1,135–137] Marked fissuring, ulceration, and crusting of footpads have also been noted in dogs with SND. Non-neoplastic differentials for SND include hepatic disease and severe diabetes mellitus. Necrolytic migratory erythema, diabetic dermatopathy, and hepato-cutaneous syndrome are terms that have been previously used to describe SND. This PNS resolves in people after surgical extirpation of the glucagonoma; however, the prognosis appears poor in dogs due to multiple postoperative complications.[138] Though not specifically related to hepatocutaneous syndrome, dogs with sarcomatoid renal cell carcinoma and paraneoplastic hepatopathy with similarities to human Stauffer's syndrome have been reported.[137,139]

Miscellaneous Syndromes

The number of recognized cutaneous PNS in humans easily reaches into the dozens; however, the specificity of which we are able to delineate similar PNS in veterinary oncology is limited.[116] Ischemic necrosis of the digits or feet is a common human PNS associated with lymphoma, adenocarcinoma, and occasionally other malignancies.[140] Symmetric cutaneous necrosis of the hind feet has been reported in a cat with multicentric follicular lymphoma.[141] Interestingly, the necrosis was not associated with a neoplastic infiltrate, and thrombotic/vasculitic causes were not seen histologically, suggesting this case may be of paraneoplastic origin.

Malassezia-associated dermatitis was first reported in a cat with paraneoplastic alopecia from a metastatic exocrine pancreatic carcinoma.[142] A study of more than 500 feline skin biopsies found 15 (2.7%) of the submissions contained Malassezia organisms. Ten of the 15 cats also had neoplasia, suggesting that Malassezia yeast in feline skin biopsies should prompt a clinical workup for neoplasia.[143]

Pemphigus vulgaris (PV) is a dermatopathy characterized by intraepidermal bullae and erosions of the skin and oral mucosa. In humans, a paraneoplastic PV (PPV) can be associated with lymphoma, Kaposi's sarcoma, and various carcinomas.[1] A 7-year-old female Bouvier was reported to have PPV in association with mediastinal lymphoma.[144] In addition, an 8-year-old horse was reported to have paraneoplastic pruritis and alopecia in association with lymphoma and hypercalcemia of malignancy.[145] Canine PPV may be a comparative model to human PPV.[146] The variety noted in these reports should serve as a reminder that there are very likely many other uncharacterized-to-date cutaneous PNSs in veterinary oncology.

RENAL MANIFESTATIONS OF CANCER

Human and veterinary cancer patients can develop important renal complications. Many are iatrogenic in nature and include chemotherapy-related toxicity (e.g., cisplatin; see Chapter 11), antibiotic toxicity (e.g., aminoglycoside), contrast-associated nephropathy, and others. In addition, infiltrative malignancies such as lymphoma can have devastating consequences on the kidneys. Biochemical alterations leading to nephrotoxicity include tubular precipitation from hypercalcemia or protein casts, glomerulopathies due to amyloidosis or membranous glomerulopathy, and fluid and electrolyte disorders due to hypercalcemia, hyponatremia, and acute tumor lysis syndrome. Nephrogenic diabetes insipidus may be a renal PNS in dogs with intestinal leiomyosarcoma.[147]

Approximately 6% to 10% of human cancer patients have significant glomerulonephritis and protein loss in the urine.[148] Similarly, 11% of humans with nephrotic syndrome have a concurrent diagnosis of cancer.[149] The most common malignancies associated with PNS glomerulonephritis in humans are carcinomas of the lung and gastrointestinal tract.[150] Immune complexes are thought to play a central role.[151,152] The prevalence of glomerulonephritis in veterinary cancer patients is unknown; however, immune complex glomerulonephritis has been reported in a dog with polycythemia vera and a dog with lymphocytic leukemia.[153,154] Treatment involves removal of the underlying tumor.

NEUROLOGIC MANIFESTATIONS OF CANCER

Greater than 50% of human cancer patients have a mild degree of neuromuscular dysfunction (myopathy or peripheral neuropathy); however, the frequency of specific neurologic PNS is low.[1,155,156] Neurologic PNS are separated into anatomic categories (brain, spinal cord, peripheral nerve, muscle, neuromuscular junction). Their prevalence in veterinary patients is unknown.

Neurologic PNS has been reported in the dog involving the brain, peripheral nerve, and neuromuscular junction.[157–160] The astute clinician will remember that several non-PNS causes of neurologic complications such as metabolic encephalopathy, brain metastasis, cerebrovascular incidents due to coagulation defects or thrombocytopenia, neurotoxicity from radiation or chemotherapy, and neurologic infections due to altered immunity are possible.

Myasthenia Gravis

Myasthenia gravis (MG) is an acquired or congenital disorder of the neuromuscular junction that results from a failure of transmission across the neuromuscular junction. Antibodies to nicotinic acetylcholine receptors (AchRe) can be documented in dogs with MG.[157] The detection and serial monitoring of AChRe antibodies are useful in the diagnosis and follow-up of canine MG. In a similar fashion, Lambert-Eaton syndrome in humans occurs due to calcium-channel autoantibody formation from lung tumors, which then results in an MG-like syndrome due to poor calcium influx from the presynaptic neuromuscular junction.[163,164]

The most common cause of acquired MG in the dog is thymoma; however, it has also been reported in association with osteosarcoma, lymphoma and bile duct carcinoma.[158, 165–170] The clinical signs of MG revolve around intermittent muscular weakness, which translates to exercise intolerance, dysphagia and megaesophagus (and possible secondary aspiration pneumonia). Treatment involves removal of the inciting tumor (see Chapter 32, Section B); rapid clinical improvement and decreases in AchRe antibodies have been noted after surgical extirpation of thymoma.[166] The use of immunosuppressive doses of prednisone (greater than 2 mg/kg PO daily) may also be a useful adjunct.[167]

Peripheral Neuropathy

Peripheral nerve lesions due to cancer are a common event in humans and in animals. When nerve fibers were analyzed from dogs with a wide variety of malignancies, abnormal findings such as demyelination, myelin globulation, and axonal degeneration were noted in some specific malignancies.[159] Tumors in dogs associated with peripheral nerve changes include primary lung tumors, insulinoma, mast cell tumor, thyroid adenocarcinoma, melanoma, mammary tumors, leiomyosarcoma, undifferentiated sarcoma, hemangiosarcoma, and multiple myeloma.[159–161,171,172] Clinically apparent PNSs of the peripheral nerves in veterinary medicine are rare. A multisystemic PNS in people secondary to plasma cell dyscrasia or tumor is termed POEMS syndrome, for polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes. The treatment for paraneoplastic peripheral neuropathy is removal of the inciting tumor.

Diencephalic Syndrome

Diencephalic syndrome is a PNS seen with rostral hypothalamic tumors in infants that undergo extreme emaciation despite normal to increased caloric intake.[1] All cases occur in association with a tumor in the diencephalic region that secretes excess growth hormone. Only one case has been reported in the veterinary literature (affecting a 3-year-old Doberman pinscher).[162] This dog had extreme emaciation in the face of increased caloric intake with a growth-hormone producing astrocytoma in the diencephalic region. The cause for the lack of acromegalic and accelerated growth signs in this case is unknown.

MISCELLANEOUS MANIFESTATIONS OF CANCER

Hypertrophic Osteopathy

Hypertrophic osteopathy (HO) is a syndrome characterized by periosteal proliferation of new bone along the shafts of long bones in response to malignant and nonmalignant diseases (Figure 5-4). HO has been noted in the medical literature for more than 25 centuries as Hippocrates described "Hippocratic fingers" (digital clubbing seen in humans with HO). As a PNS, HO is most commonly due to primary lung tumors.[1,173] HO has also been reported in dogs with tumors metastatic to the lungs, urinary bladder rhabdomyosarcoma, esophageal tumors, malignant Sertoli cell tumors, renal transitional cell carcinoma (TCC), and nephroblastoma, and in cats with renal papillary carcinoma or adrenocortical carcinoma.[91,174–181] Nonmalignant conditions such as heartworms, heart disease, focal lung atelectasis, pregnancy, abscesses, granulomas, foreign bodies, and pneumonia have also been associated with HO.

The clinical signs of dogs or cats afflicted with HO typically include a history of shifting leg lameness or reluctance to move with all four limbs affected. The limbs are typically warm to the touch and swollen with occasional cases having involvement of the ribs or pelvis. The diagnosis of HO is made by radiography of the affected bones (starts distally and moves proximally) and finding the unique 90-degree periosteal reaction seen in Figure 5-4. The search for the inciting tumor begins with radiographs of the thorax and, if negative, radiographs and/or ultrasound of the abdomen.

The etiology of HO is unknown; however, with the increased periosteal blood flow and resultant connective tissue and periosteal proliferation seen in this PNS, it has been theorized that HO develops as a result of afferent neurologic stimulation.[182] This theory is further

Figure 5-4

Hypertrophic osteopathy in the front limb of a dog with a primary lung tumor. This paraneoplastic syndrome is a bony disease that results in periosteal proliferation and subsequent lameness. This syndrome is seen in a wide variety of malignant and nonmalignant diseases.

supported by resolution of HO after vagotomy. Vagotomy is not routinely used for HO.[183] Some research suggests that excess production of growth hormone releasing hormone (GHRH) or vascular endothelial growth factor (VEGF) by the tumor may also contribute to HO.[184,185] Treatment involves removal of the inciting tumor when possible, and reports have documented resolution of HO in dogs treated for their primary tumor.[186,187] Prednisone (1 to 2 mg/kg PO daily) or nonsteroidal antiinflammatory drugs (NSAID) may be a useful adjunct therapy for HO when the inciting tumor cannot be removed (i.e., diffuse metastasis). Other treatments for HO such as intercostal nerve resection, unilateral vagotomy, bilateral cervical vagotomy, analgesics, and subperiosteal rib resection have been suggested; however, these therapies have not been evaluated extensively in veterinary patients.[173,188] The use of bisphosphonates has become more common for human HO[189] and likely represents an exciting new therapeutic modality in veterinary medicine for a variety of indications.[43,44]

Fever

Though the most common causes of fever are infection, inflammation, autoimmune disease, or drug/blood product reactions, cancer can cause fever as a PNS and should always be considered as an important differential. Paraneoplastic fever can accompany a wide variety of tumors in human and veterinary patients. The incidence of fever as a PNS in veterinary medicine is unknown; however, in human patients presenting for fever of unknown origin, cancer (lymphoma, hepatoma, and renal cell carcinoma most commonly) is the cause of in over one third of the cases.[1] Approximately 10% of human cancer patients develop noninfectious/inflammation-related fever at some point during the course of their disease.[190] The pathogenesis of PNS fever is predominately due to the excess production of cytokines (IL-1, IL-6, TNF-alpha, and interferons) and febrile-promoting prostaglandins.[1,190,191]

The most important point in managing fever in veterinary patients with cancer is the evaluation for the presence of infection. Cancer patients with neutropenia and fever represent a medical emergency. Cancer patients without neutropenia and fever are not medical emergencies but must still be worked up to determine the presence or absence of an infectious or inflammatory nidus. If one is not found, then PNS fever is more likely the cause. If the fever is severe and threatens quality of life, the use of an NSAID such as indomethacin or naproxen is commonly utilized in humans with PNS fever.

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Diagnostic Imaging in Oncology

Lisa J. Forrest

Diagnostic imaging plays an essential role in the management of the cancer patient. The initial diagnosis, staging, surgical and radiation treatment planning, and response to therapy all involve imaging to a varying extent. Routine radiographs, ultrasound, nuclear medicine, and cross sectional imaging in the form of computed tomography (CT) and magnetic resonance imaging (MRI) are routinely used in veterinary oncology. The choice of imaging modality depends on many factors, including the desired outcome. The biologic behavior of the tumor directs the imaging choice in cancer staging, and imaging may play an important role in guiding serial tumor biopsy during the course of therapy. The sophistication of imaging modalities continues to increase exponentially. Each modality has advantages and disadvantages with regard to cost, availability, sensitivity, specificity, and qualities of anatomic versus functional imaging (Table 6-1). Advanced molecular imaging techniques, which measure biologic processes at the cellular level,[1] are quickly becoming commonplace in physician-based oncology and have the potential to play an important role in the tailoring of cancer therapy in veterinary patients.

IMAGING MODALITIES

Radiography

Conventional radiography has been the mainstay of cancer imaging for many years because of its accessibility and low cost. However, it usually is relegated to a screening test and often followed by other imaging modalities to better differentiate and define tumor extent and to evaluate for pulmonary metastases (Figure 6-1). Radiographic images are produced by the differential absorption of x-rays as the primary beam passes through the patient. Some x-ray photons are absorbed by the body, and some pass through it. Absorption depends on the thickness, physical density, and effective atomic number of the tissues of the patient's body. The x-rays that are not absorbed reach the radiographic film and determine the blackness and gray scale of the image.

Two of the strengths of radiography are the global information it provides and its excellent utility for bone imaging, especially of the appendicular skeleton (Figure 6-2). Thoracic and abdominal radiographs are excellent screening tools for feline and canine lymphoma patients to determine thoracic lymph node and pulmonary involvement, as well as liver, spleen, and abdominal node neoplastic spread. Radiography's greatest weakness is the superimposition of overlying structures. CT and MRI have largely supplanted radiography for imaging of the head and, in some circumstances, the axial skeleton.[2-4]

Computed Tomography

As does radiography, CT relies on the physical density differences between tissues to form the image. Unlike radiography, CT portrays *slices* of the patient without superimposition of structures; because the images are computer generated, the gray scale display is superior with CT. Although thoracic radiographs are routinely used as a screening method to evaluate primary and metastatic tumors of the lung, mediastinum, and ribs, CT is the superior imaging modality for these structures (Figure 6-3).[5-7]

CT is exceedingly useful for both surgical and radiotherapy treatment planning. Although MRI has better tissue differentiation, CT is the better modality for radiotherapy treatment planning because there is no image distortion and the physical tissue density is available for input into treatment planning computers.[8,9] It is extremely important to position the radiotherapy patient for the planning CT in a way that can reliably be repeated during therapy. This ensures that the treatment is delivered as planned.

Compared with radiography, CT is more sensitive for identifying pulmonary nodules, mediastinal lymphadenopathy,[5,6] and mediastinal and pleural masses (Figure 6-4).[10] Primary lung tumors should be imaged with CT to evaluate for intrathoracic metastases and tracheobronchial lymphadenopathy (Figure 6-5).

TABLE 6-1   **Comparison of Imaging Modalities Used in Veterinary Medicine**

Modality	Cost	Sensitivity	Specificity	Availability
Radiography*	Low	Moderate-high	High	High
Ultrasound[†]	Moderate	High	Low-moderate	Moderate-high
CT[†]	High	Moderate-high	Moderate-high	Moderate-high
MRI[†]	High	High	Moderate-high	Moderate-low
NM[†]	Moderate-high	Moderate-high	Low	Moderate-low
SPECT and PET[†]	High	High	Moderate	Low
PET/CT[†]	High	High	High	Low

CT, Computed tomography; MRI, magnetic resonance imaging; NM, nuclear medicine; SPECT, single photon emission computed tomography; PET, positron emission tomography.

*Anatomic imaging.

[†]Functional/physiologic imaging.
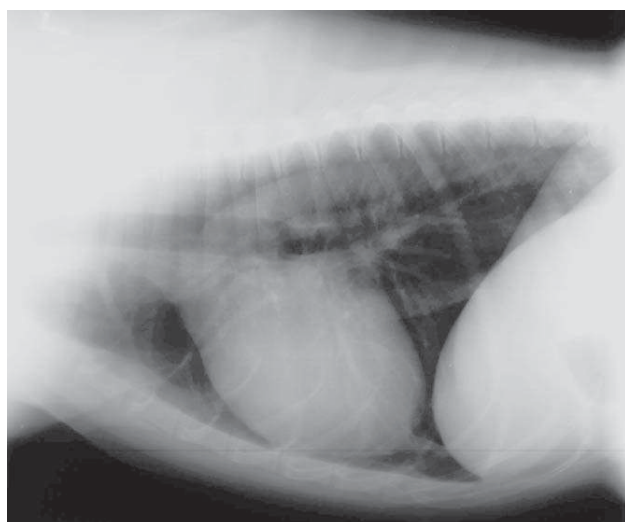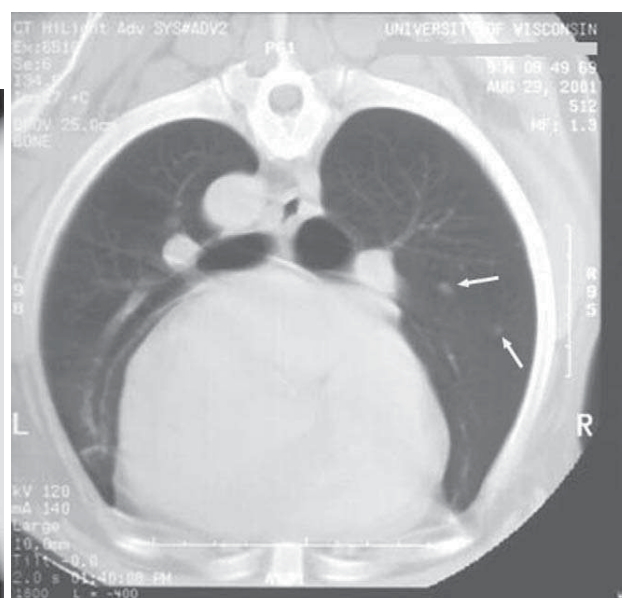
A   B

Figure 6-1

Lateral thoracic radiograph **(A)** and corresponding computed tomography (CT) view **(B)**. Note the small pulmonary metastases *(white arrows)* identified on CT, which were not seen on survey thoracic radiographs.

CT-guided biopsy can be done if masses are not visualized with ultrasound (Figure 6-6).[11-14] Other sites, especially the skull, are best imaged with CT (Figure 6-7),[2,15-19] although MRI is better suited for neuroimaging.

Contrast-enhanced CT imaging increases lesion conspicuity and the extent of tumor infiltration. Contrast-enhanced CT images are invaluable for detecting tumor extension associated with soft tissue sarcomas, especially feline vaccine-associated sarcomas.[20] Feline vaccine-associated sarcomas are routinely imaged with CT for both surgical and radiotherapy planning (Figure 6-8). Wash-in and wash-out techniques have been used to estimate tumor perfusion, vascular permeability, and tumor blood volume as a means of predicting tumor aggressiveness.[18,21]

Ultrasonography

Ultrasound imaging's ability to evaluate the internal structure of organs has made it an essential diagnostic

Figure 6-2

Lateral radiograph of a dog with a distal radial osteosarcoma. Note the moth-eaten lysis within the radial metaphysis and the irregular periosteal proliferation caudally.

tool that has replaced abdominal radiography as the first-line choice in the evaluation of the abdomen. The information provided by ultrasound is based on differences in acoustic impedance. The acoustic impedance of a material is a product of its physical density and the velocity of sound in the material. As sound waves pass from tissue to tissue, the amount of sound reflected (echoes) is determined by the impedance difference between tissues. The reflected echoes are detected by the transducer and processed into an image.

Ultrasound images are superior to radiographs in cases of pleural or peritoneal effusion because of the loss of visceral detail that occurs on radiographs as the fluid silhouettes with organs. In a recent retrospective study, ultrasound proved useful in the diagnosis of carcinomatosis in cats. In all 14 cats, peritoneal effusion and peritoneal masses were detected sonographically.[22] Ultrasound is also useful for guiding a biopsy or fine-needle aspiration of an abnormality. It is more sensitive than survey radiographs for detecting abdominal

lymphadenopathy (Figure 6-9),[23-25] although radiographs are necessary to detect any bony invasion that may be associated with malignant medial iliac lymph nodes. Ultrasound is sensitive in the detection of adrenal abnormalities, and vessel invasion, usually associated with malignancy, can be identified (Figure 6-10).[26] Ultrasound is routinely used to stage and monitor cats and dogs with mast cell disease, which can have a varied appearance (Figure 6-11).[27,28] It is used extensively in patients with gastrointestinal disease for initial diagnosis and for monitoring therapy (Figure 6-12).[25,29-32]

Ultrasonography is sensitive for lesion detection, but it is not specific for disease etiology. Many studies have attempted, unsuccessfully, to differentiate benign from malignant lesions based on sonographic appearance.[26,28,31,33-35] Therefore biopsy or fine-needle aspiration of lesions is necessary. Ultrasound-guided sampling of tissue generally can be performed quickly, accurately, and safely.[36-40] A caveat is the potential seeding of tumor cells from ultrasound-guided percutaneous sampling of transitional cell carcinoma.[41]

Advances in ultrasound equipment and the development of sonographic contrast agents are increasing the specificity of ultrasound.[42] With Doppler techniques, tumor vasculature can be assessed and is generally tortuous and of higher velocity compared with normal tissues.[43] Tissue harmonic imaging, a new technique, transmits at one frequency and receives at twice that frequency. This technique has advantages over fundamental imaging.[42,44] The use of harmonic imaging or ultrasound contrast agents (or both) has the potential to allow differentiation of benign from malignant lesions[44-49] and warrants continued investigation.

Magnetic Resonance Imaging

As does CT, MRI produces slices in the transverse, sagittal, and dorsal planes. However, CT images in the sagittal and dorsal planes are reconstructed from the transverse images, whereas the different image planes obtained with MRI are acquired in real time, not reconstructed; MRI is a true three-dimensional imaging modality. MRI is an advanced imaging technique based on the properties of hydrogen atoms placed in magnetic and radiofrequency fields. The primary use of MRI in veterinary medicine is evaluation of the central nervous system (Figure 6-13). Brain imaging with MRI provides better anatomic detail than CT, but because of the number and characteristics of hydrogen protons in bone, MRI is less useful for assessment of cortical bone. MRI provides excellent anatomic detail of soft tissues and is useful for the evaluation of joints, tendons, muscles, and bone marrow of the musculoskeletal system.

MRI is the imaging modality of choice for the central nervous system. It is an excellent modality for brain and spinal tumors and tumors of the brachial

A   B
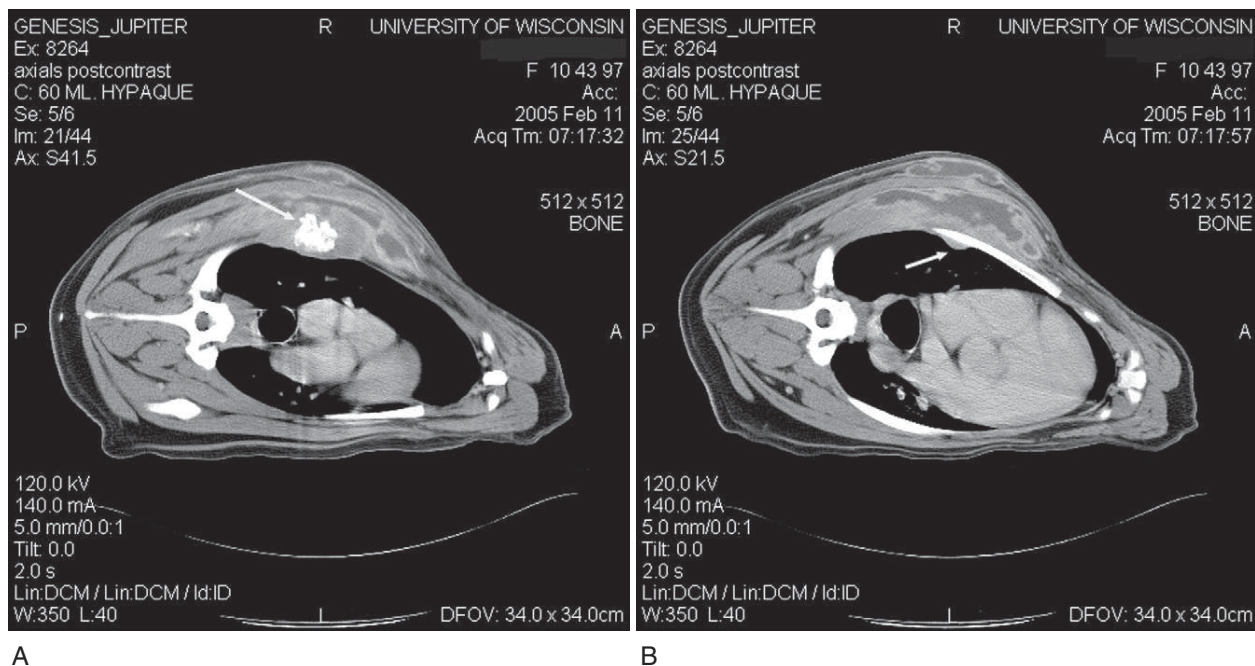
Figure 6-3

CT images of a chondrosarcoma of the rib after contrast administration. **A**, Note the bony proliferation *(white arrow)* of the rib and **(B)** the considerable soft tissue and pleural extent *(white arrow)* of the mass.

A   B
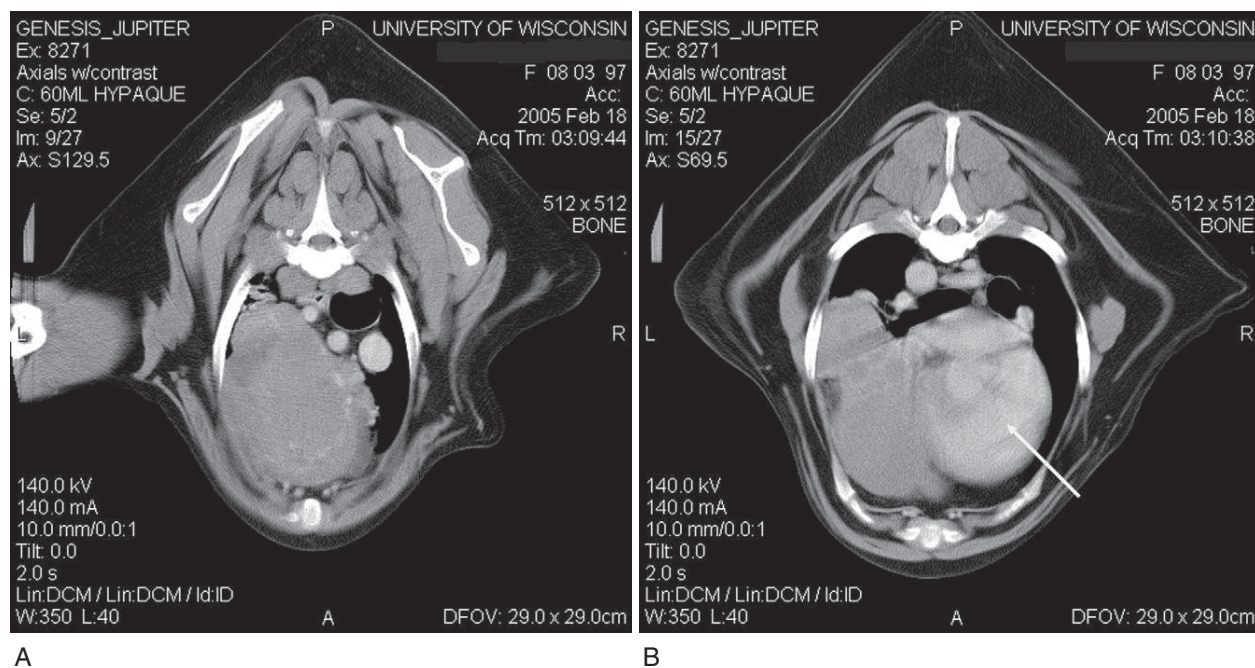
Figure 6-4

CT images of a thymoma after contrast administration. **A,** Note the large soft tissue attenuating mass with minimal enhancement in the cranial mediastinum. **B,** On a transverse image caudal to the figure shown in **A,** deviation of the heart *(white arrow)* to the right, caused by the thymoma, can be seen.
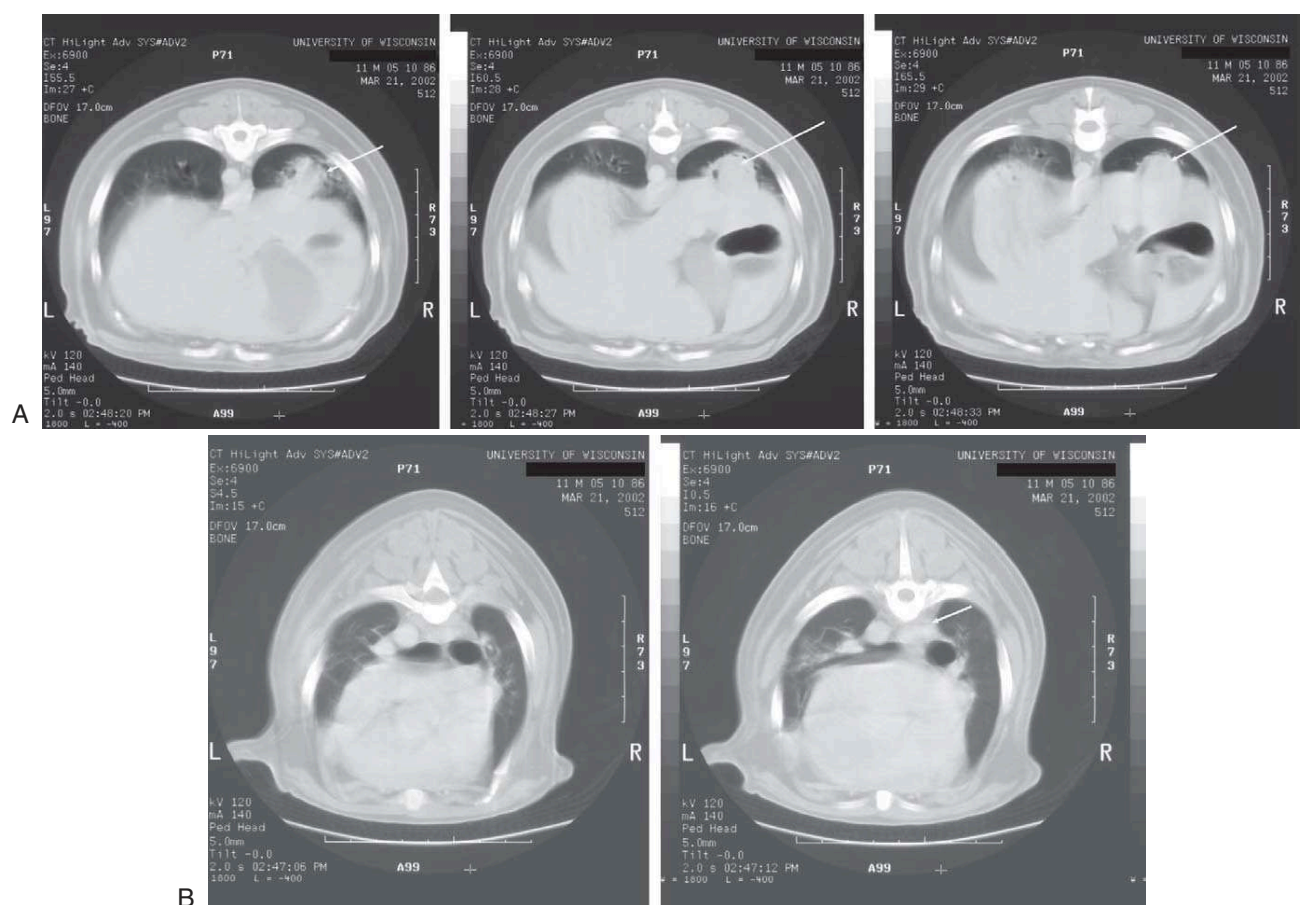
Figure 6-5

A, Three consecutive CT images, obtained after contrast administration, of a primary lung carcinoma in the right caudal dorsal lung *(white arrows)* of a dog. **B,** Evidence of enlargement of the tracheobronchial lymph nodes *(white arrow)*, which is suggestive of metastasis, can be seen.

plexus (Figure 6-14). As advanced MRI equipment and techniques (e.g., dynamic contrast-enhanced MRI) make their way into veterinary practice, they will find additional uses, including evaluation of functional changes in tissue (e.g., perfusion and blood flow). MRI already has been shown to be more sensitive for determining the soft tissue tumor extent of appendicular osteosarcoma,[50] invaluable information for limb-sparing procedures.

Nuclear Medicine

Diagnostic nuclear medicine, or scintigraphy, involves the administration of radiopharmaceuticals that localize to an area of interest in the body by physiologic processes. Images obtained from nuclear medicine studies do not provide the anatomic detail attainable with other imaging techniques; however, the functional dependence on physiologic processes adds important information. Technetium-99m (^{99m}Tc) is the most commonly used radionuclide because it has excellent imaging qualities and a short half-life (6 hours) and is easily bound to localizing pharmaceuticals. Bone scintigraphy using ^{99m}Tc methylene diphosphonate (^{99m}Tc-MDP) is a nuclear medicine study that is frequently used in veterinary medicine because it is a simple, sensitive, and noninvasive method of evaluating the entire skeleton.[51] Other commonly used nuclear imaging studies are renal, thyroid, lung, and liver scintigraphy (Figure 6-15).

In general, nuclear medicine studies are sensitive for lesion detection but are nonspecific for disease etiology. Benign and malignant lesions can have a similar scintigraphic appearance. Bone metastases can be detected earlier with scintigraphy, before clinical and radiographic recognition, and scintigraphy can aid the choice of the best site for biopsy (Figure 6-16).[51,52,52b] Once a lesion has been identified, additional imaging (radiography,

A

B
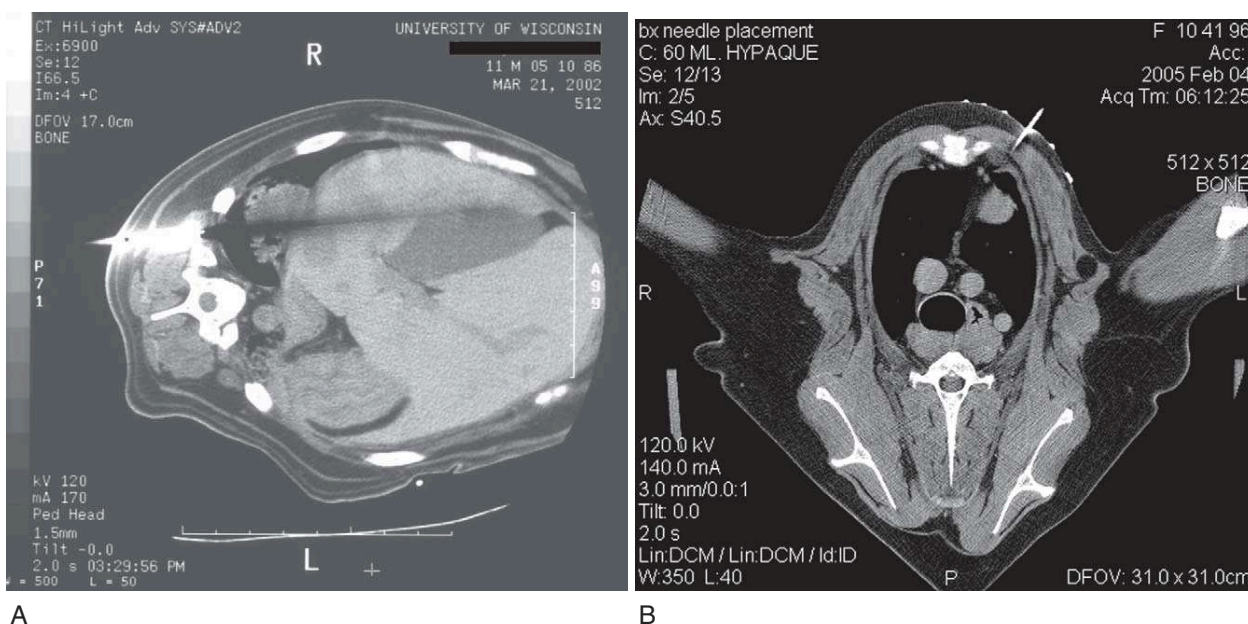
Figure 6-6

A, CT-guided biopsy of a lung mass in the same patient as in Figure 6-5. The metallic biopsy needle *(white arrow)* is within the dorsal subcutaneous tissues and creates a metal streak artifact *(black line)* distal to the needle. **B,** CT image for needle placement before CT-guided biopsy of a sternal lymph node that could not be seen with ultrasound. The white marks on the skin are barium paste, used for localization purposes.

Figure 6-7

CT image, after contrast administration, of a cat with a nasal carcinoma. The image is at the level of the eyes, and the tumor is primarily left sided. Note the soft tissue attenuating mass in the left nasal cavity that extends into the nasopharynx and frontal sinus, with evidence of bone destruction *(white arrow).*

ultrasonography) may be necessary. A nuclear medicine department requires expensive equipment, a radiopharmacy, and personnel trained in the safe handling of radiopharmaceuticals.

SPECT and PET

Two advanced radionuclide imaging techniques used extensively in human medicine are single photon emission computed tomography (SPECT) and positron emission tomography (PET). SPECT uses traditional gamma ray–emitting radionuclides, often with a rotating gamma camera or stationary ring that reconstructs the images in cross sections. SPECT provides better lesion localization than planar scintigraphy. PET detectors also perceive gamma rays in a cross section to reconstruct a three-dimensional image, but these gamma rays are produced during annihilation of another emitted particle, the positron.[53,54] Positron-emitting radionuclides are closer in identity to physiologic atoms than are gamma ray–emitting radionuclides, allowing synthesis of compounds of more biologic relevance.[53,55] Combination PET/CT scanners have been developed and are commercially available. The two systems are housed in the same gantry, allowing for almost simultaneous acquisition of both images and their fusion, providing both anatomic and physiologic information. To date, the complementary data provided by the two imaging procedures provide the
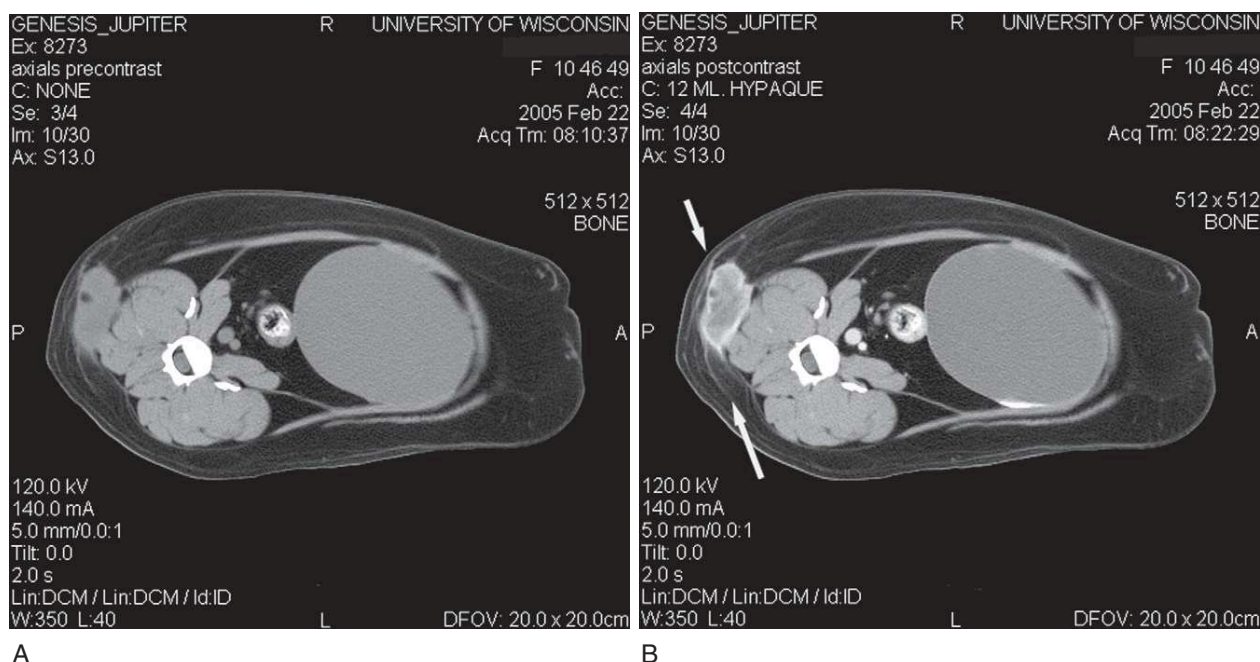
Figure 6-8

CT images of a cat with a vaccine-associated sarcoma in the subcutaneous tissues overlying the lumbar spine. **A,** A transverse image obtained before administration of an intravenous contrast medium. **B,** The same CT slice after contrast administration. Note the contrast enhancement of the mass, as well as enhancement extending along fascial planes *(white arrows)*.
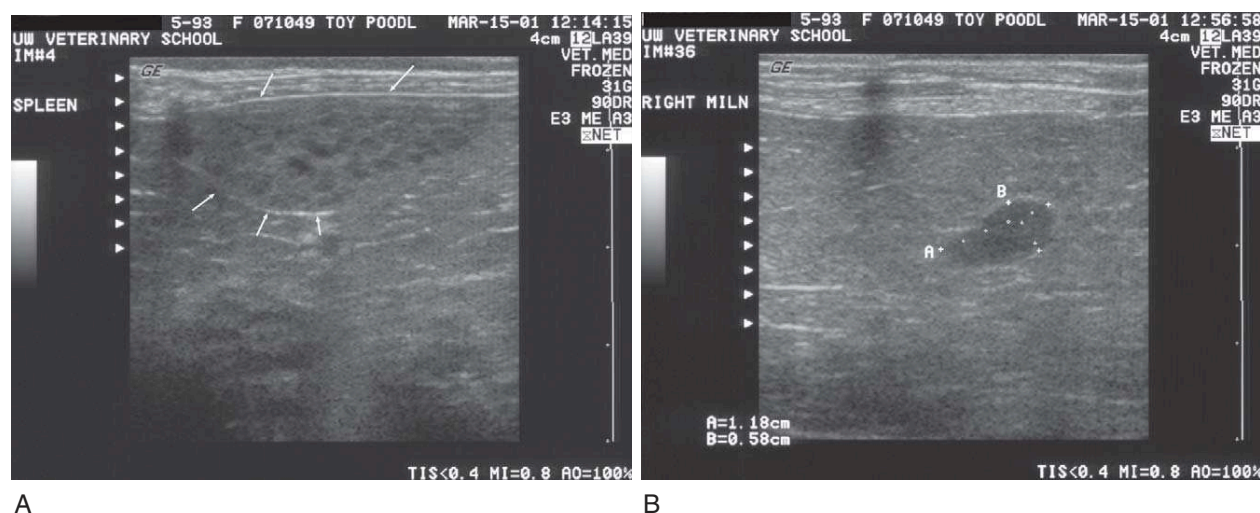
Figure 6-9

Ultrasound images of a dog with lymphoma involving the spleen **(A)** and medial iliac lymph node **(B)**. In **A** the spleen is outlined by white arrows; note the hypoechoic nodules throughout the spleen.

most accurate information available for oncology patients.[56-59]

Compared with most normal cells, tumor cells have increased glucose utilization through glycolysis; this makes them molecules that are appropriate for modification and labeling with a positron-emitting radionuclide.[60] Tumor-increased energy demand is met through upregulation of the hexose monophosphate pathway, cell membrane glucose transporter proteins, and hexokinase. A glucose analog, ^{18}F-fluorodeoxyglucose (FDG), acts as a glucose

Figure 6-10

Ultrasound images of a right adrenal pheochromocytoma with invasion into the right renal vein. **A,** Note the large heterogeneous mass cranial and medial to the right kidney (*RK*). **B,** Note the turbulent Doppler flow in the right renal vein, indicating invasion of the right adrenal mass (*RA*). Tumor invasion of the caudal vena cava also had occurred, and a tumor thrombus was present.

Figure 6-11

Ultrasound images of a cat with a mast cell tumor of the spleen. **A,** Normal image of the liver. **B,** The spleen is abnormally large and shows decreased echogenicity; it is isoechoic to hypoechoic compared with the liver.

molecule, is transported by membrane proteins, and is phosphorylated by hexokinase in the cell. However, phosphorylated (FDG [18F]-fluordeoxyglucose) is not used in the glycolytic pathway and remains trapped intracellularly, because tumor cells are deficient in glucose-6-phosphatase. The differential uptake of FDG by tumor cells forms the basis for one type of contrast in PET images.[61]

FDG imaging has been used extensively for human cancer imaging.[62] With whole-body imaging, as is done in human scans, it is especially useful for detecting distant metastases.[62] However, there are limitations to the interpretation of FDG PET images. Increased FDG uptake occurs with inflammation, often seen early after radiation therapy,[63] resulting in a false positive finding.
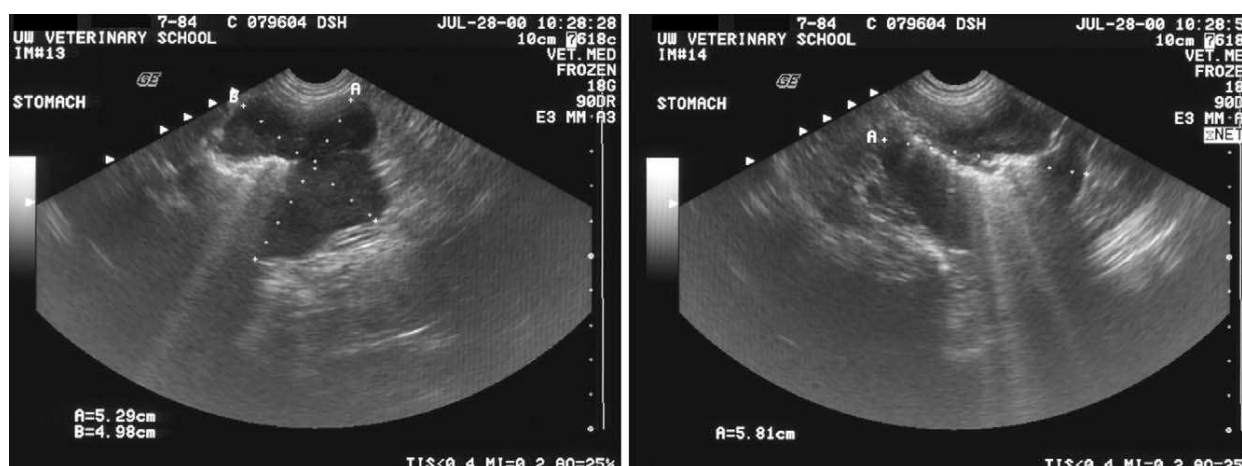
Figure 6-12

Ultrasound image of a cat with gastric lymphoma. The gastric wall is focally thick, averaging 5 cm (0.5 cm is normal), and there is complete obliteration of the normal gastric layering.

A   B
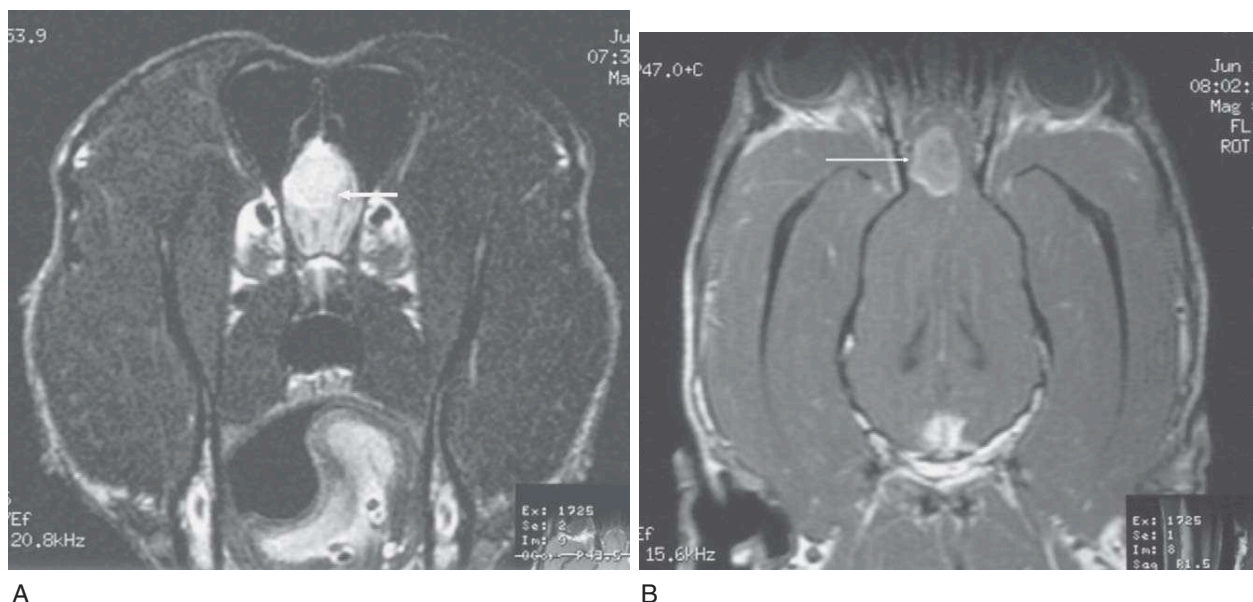
Figure 6-13

Magnetic resonance images of a dog with a meningioma of the left frontal lobe. **A,** On a T2-weighted image, the mass is hyperintense to surrounding brain *(white arrow)*. **B,** A dorsal plane T1-weighted image, after gadolinium administration, shows intense contrast enhancement of the mass that arises from the falx *(white arrow)*.

Certain low-grade or poorly differentiated tumors do not show sufficient FDG uptake to allow them to be distinguished from background.[60] Normal gray matter of the brain can confound interpretation of intracranial tumor images because of its normally high glucose utilization.[60]

The limitations of FDG PET imaging have led to exploration of other imaging agents. Thymidine, the only nucleoside used solely in the construction of deoxyribonucleic acid (DNA) and not in ribonucleic acid (RNA),[64] provides an accurate measurement of increased cellular proliferation in a tumor both before and after treatment. Two fluorinated compounds that have proved stable to degradation and useful in tumor imaging are 2′-fluoro-5-methyldeoxyuracil (FMAU)[65] and 3′-deoxy-3′fluorothymidine (FLT).[66] Both of these compounds are phosphorylated by thymidine kinase (TK) intracellularly and incorporated into DNA to variable degrees. Although FLT acts as a chain terminator in DNA synthesis and has limited incorporation into DNA, for imaging purposes it remains trapped intracellularly by TK and indirectly reflects cellular proliferation.[57]

A   B
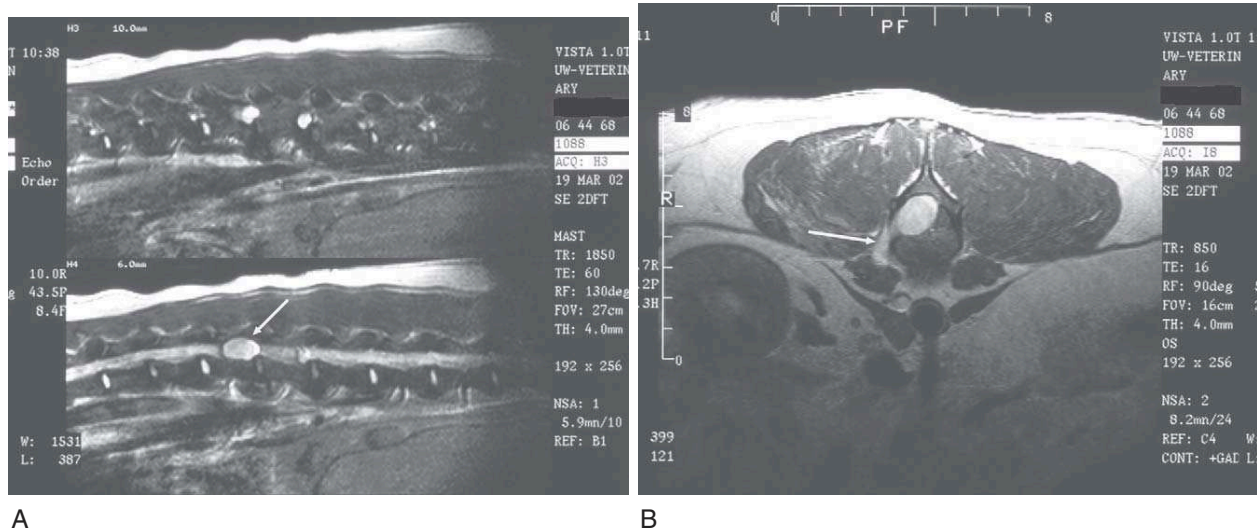
Figure 6-14

Magnetic resonance images of a dog with a nerve root tumor. **A,** On a sagittal plane T1-weighted image, after gadolinium administration, a contrast-enhancing, hyperintense mass *(white arrow)* is seen in the lumbar spine at L5-6. **B,** On a transverse plane T1-weighted image, after gadolinium administration, the contrast-enhancing mass is seen to involve the abnormally enhancing nerve root *(white arrow)*.
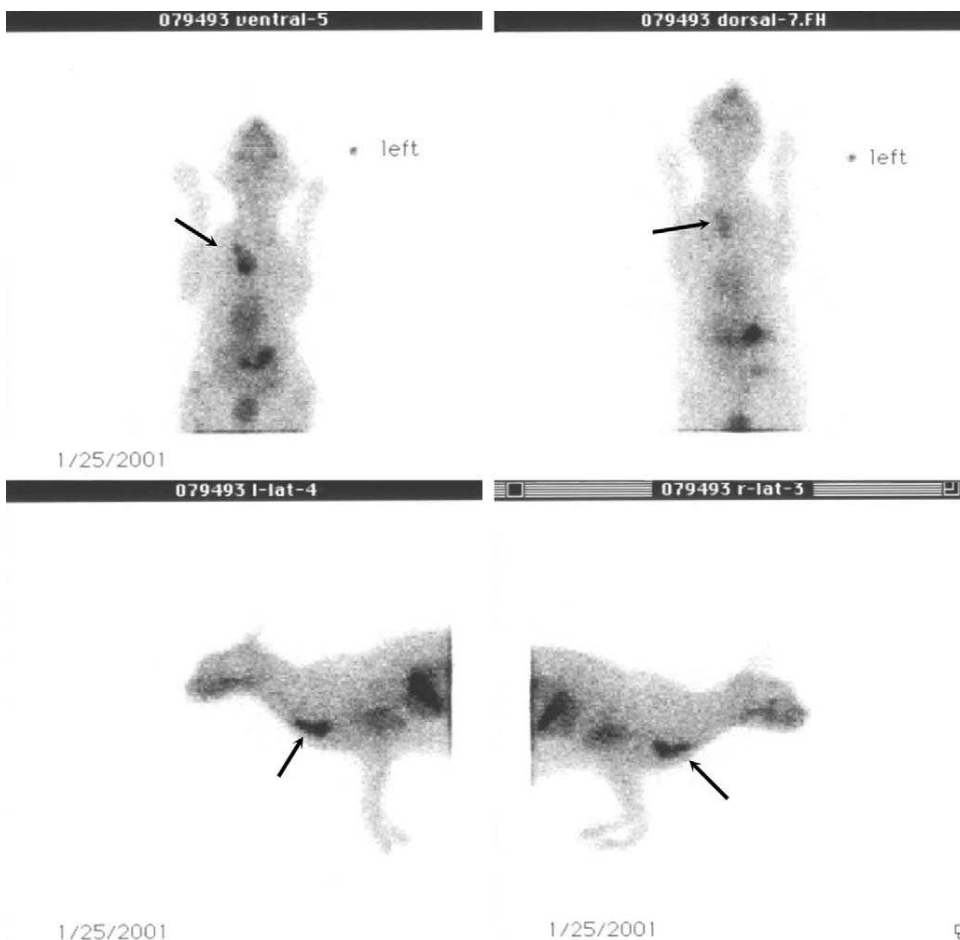
Figure 6-15

Ventral, dorsal, and left and right lateral scintigraphic images of a cat with ectopic thyroid carcinoma *(black arrows)*. The images were obtained 20 minutes after intravenous administration of technetium-99m.
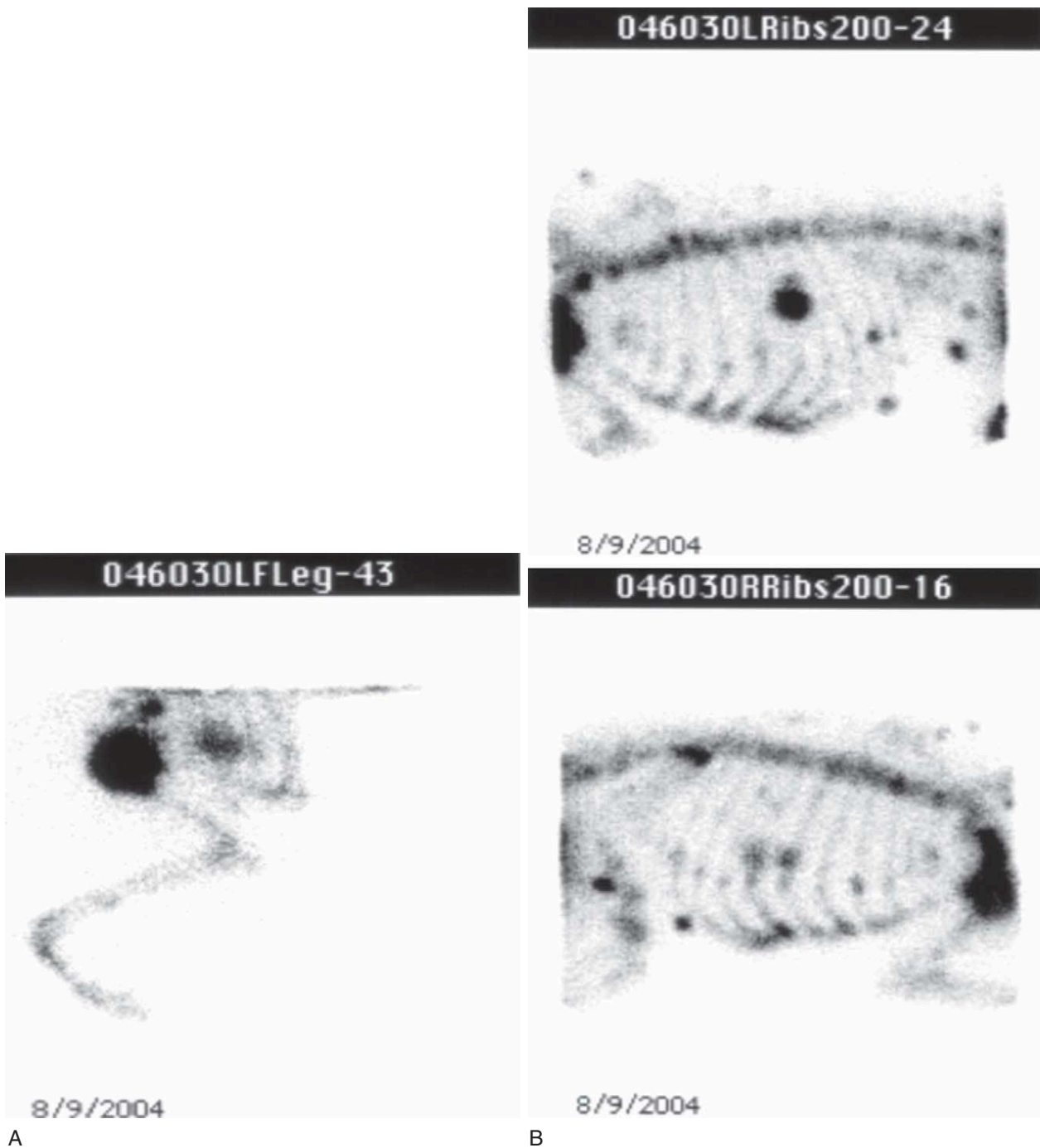
Figure 6-16

Bone scan images of a dog with primary osteosarcoma of the left proximal humerus **(A)** with subcutaneous and rib metastases **(B)**. The images were obtained 2 hours after intravenous administration of technetium-99m (^{99m}Tc) methylene diphosphonate. Note the large mass with associated radiopharmaceutical uptake in the left proximal humerus **(A)** and the focal increased uptake in multiple ribs and in subcutaneous tissue overlying the thoracic spin and dorsal to the scapula **(B)**. Note that soft tissue metastases do not always reliably uptake ^{99m}Tc.
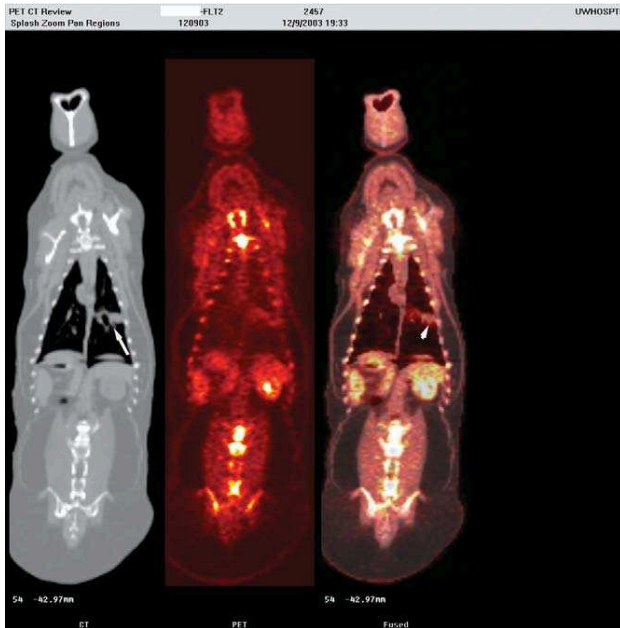
Figure 6-17

Dorsal plane PET/CT images of a dog with bronchoalveolar carcinoma of the right caudal dorsal lung after administration of FLT. The image on the left is the CT image, the middle image is the FLT PET image, and the image on the right is the fused CT/FLT/PET image. Note the cavitated mass in the right caudal lung in the CT image *(white arrow)*. Extensive FLT uptake, indicating cellular proliferation, can be seen on the PET image. This functional information correlates well with the anatomic information on the fused image *(white arrowhead in image on the right)*. The middle (PET FLT) image shows increased FLT uptake in the bone marrow of the cranial thoracic spine, the lumbar spine, and the pelvis and in the right renal pelvis. The renal pelvis uptake occurred secondary to excretion of the radiopharmaceutical.
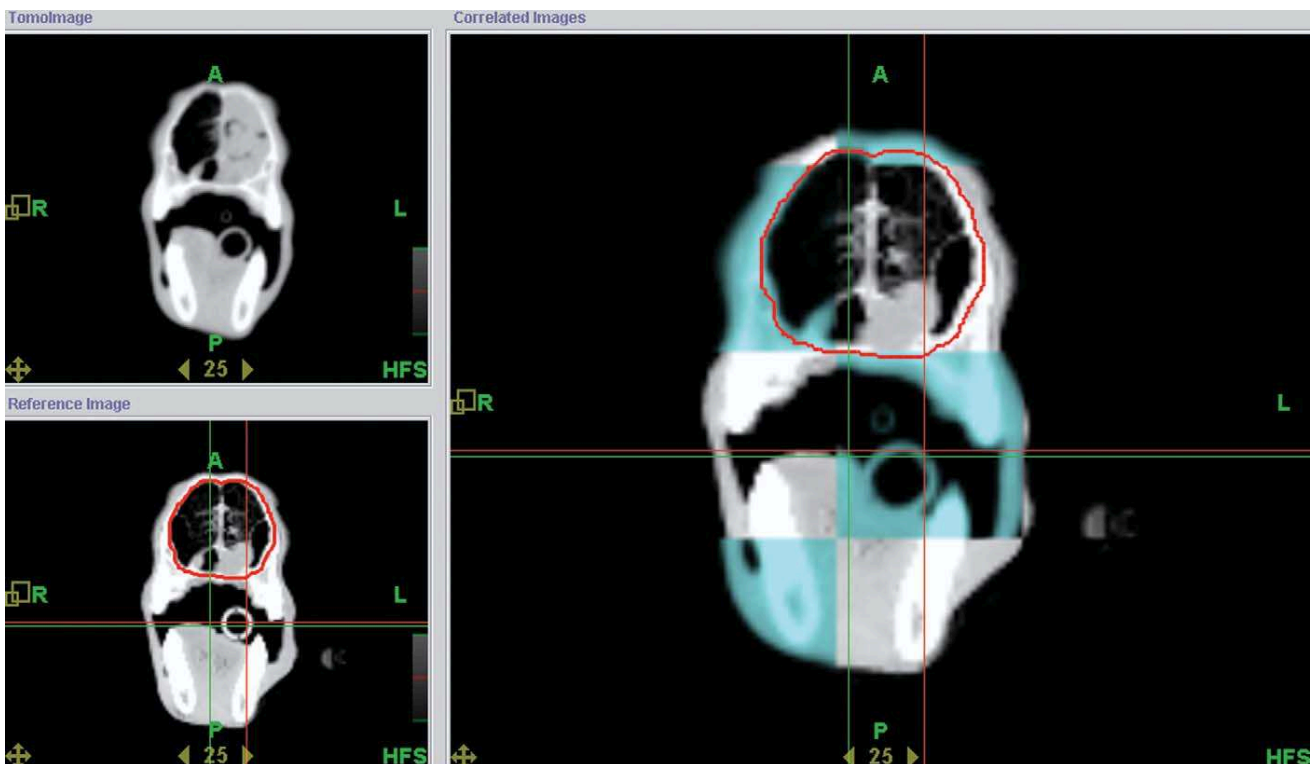
Figure 6-18

Megavoltage computed tomography (MVCT) image of a nasal tumor *(top left)* obtained with the tomotherapy machine is aligned with the planning kilovoltage computed tomography (KVCT) image *(bottom left)*, resulting in the correlated image *(large image right)*. The turquoise squares represent the MVCT image obtained before treatment; the grey squares are the planning kVCT image.

Early studies show normal FLT distribution in the bone marrow, liver, and urinary system as a result of excretion.[66] Research using dogs with spontaneous tumors continues to focus on the utility of FLT as a marker of tumor proliferation. Figure 6-17 shows a PET FLT scan of a dog with a bronchoalveolar carcinoma obtained 1 year after palliative radiation.

ADVANCES

The state-of-the-art in human radiotherapy is represented by conformal radiotherapy and intensity-modulated radiotherapy (IMRT). In the former technique, the treatment beam is conformed to the tumor volume. In the latter technique, in addition to shaping the treatment beam to the tumor volume, beam intensity is modulated to maximize tumor dose homogeneity and minimize the dose to normal tissue.[67] These advanced treatment modalities require CT imaging. Tumor motion can be a problem for many sites, especially as oncologists try to balance increased tumor dose with reduced normal tissue complications while attempting to improve overall survival. To this end, four-dimensional CT imaging currently is under investigation and being implemented in human radiation oncology centers.[67,68] Four-dimensional CT imaging takes respiratory motion into account in the treatment planning process and delivery. Another advance is helical tomotherapy, which integrates a linear accelerator with a helical CT scanner, allowing image-guided radiotherapy.[69] Each day before treatment, a CT scan is obtained with the linear accelerator; this scan is fused with the planning CT image, and the patient is moved accordingly to ensure precise treatment delivery (Figure 6-18).[70] CT imaging is an integral part of radiotherapy planning and delivery.

Advances are being made in the area of molecular imaging.[1] These techniques can be used to characterize and measure biologic processes, assess molecular targets, and monitor treatment at the cellular level. Molecular imaging can be used to assess gene delivery and identify marker proteins for apoptosis, angiogenesis, hypoxia, and other growth factors.[1,71] Moreover, the treatment of cancer using molecular targets holds great promise for individualized cancer therapy.[1,71,72]

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Diagnostic Cytology in Clinical Oncology

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Cytology is the examination of individual cells without regard to the architectural structure of the tissue. Although cytologic biopsy does not always replace excisional biopsy and histopathologic examination, it can serve as a rapid, inexpensive aid to help establish a diagnosis, and it is superior to histopathology for examination of some tissues (e.g., bone marrow) and certain conditions of the lymph nodes. Common sources of cytologic specimens include cutaneous and subcutaneous masses, body cavity fluids, ear exudates, lymph nodes, the prostate gland, bone marrow, conjunctiva, respiratory tract, vaginal and rectal mucosa, and urine. The body fluid most commonly examined cytologically is venous blood. Many of the cells seen in other fluids and tissues are commonly seen in blood. Cells may spontaneously exfoliate, as they do in body cavities or inflammatory exudates, or they may be mechanically removed by techniques such as aspiration, scraping, or washing.

This chapter discusses the equipment, technique of sample collection, cells commonly observed in cytologic specimens, causes of nondiagnostic specimens, criteria of malignancy, cytologic classification of neoplasia, neoplasia of intra-abdominal organs and body cavity effusions, neoplasia of bone marrow, and a broad classification of simple and difficult cytologic specimens.

The equipment and supplies needed for this process are minimal; they include 20 to 25 gauge needles, 3 to 20 ml syringes, glass microscope slides, immersion oil, hematologic stain, solvent, and a good microscope with an oil immersion objective (preferably planachromatic). Most veterinary cytopathologists prefer using hematologic stains, which are referred to as Romanowsky stains. They include Wright's, Wright-Giemsa, and Diff-Quik stains and other modifications. The objective is to obtain cells and quickly place them on a microscope slide in such a manner that they are not broken or too thick to evaluate and before they become incorporated into a clot.

SAMPLE ACQUISITION

Aspirates

Aspiration biopsies are useful for obtaining cells from masses, including lymph nodes and internal organs. They are performed with a 20 to 25 gauge needle and a 12 ml syringe. The skin is prepared as for a venipuncture unless a body cavity or joint will be entered; in those cases, a surgical preparation should be performed. The mass is stabilized with one hand to aid penetration, and the needle is directed by the other hand. After the mass is penetrated, negative pressure is applied to the syringe. One may redirect the needle and sample various portions of large masses while maintaining negative pressure. Negative pressure should be released before the needle is removed from the tissue. The needle is removed from the syringe, and air is aspirated into the syringe; the contents of the needle then are expelled onto the middle of a glass slide.

Alternatively, excellent aspirates can be obtained using the capillary method. The needle is not attached to a syringe, but rather is guided by holding the hub of the needle; the vigorous back-and-forth motion of the needle and the capillary pressure are adequate to detach cells without the application of negative pressure. The needle then is attached to a syringe containing air, and the contents of the needle are expelled onto a slide as described previously. Advantages of this method include less hemodilution of the sample and increased cellularity.

If the aspirate provides enough material, several preparations should be made. Films are made by placing a spreader slide on the surface of the drop of material, allowing the material to spread from the weight of the top slide, and then gently sliding the two slides apart. The sample is allowed to air dry and then stained.

Scrapings

Scrapings occasionally are useful for obtaining cells from firm surfaces, as may be done with conjunctival

scrapings or scrapings from firm cutaneous lesions. Scalpel blades or spatulas (commercially available for this purpose) may be used. The cells procured are gently spread on a glass slide. This method commonly results in numerous broken cells, but areas of intact cells usually can be found.

Swabs

Swabs may be used to obtain cells from mucosal surfaces, such as the vagina, rectum, or nose. After sample collection, the swab is gently rolled along the surface of a microscope slide.

Imprints

Imprints can be made of superficial cutaneous lesions or of tissues removed at surgery or necropsy. Imprints of superficial lesions may be diagnostic, but they often reflect only surface inflammation and contamination. To obtain imprints of other tissues, one should blot the freshly cut surface with absorbent paper (e.g., paper towel) to remove excess blood and tissue fluid and then gently touch the surface of the tissue with a glass microscope slide. Care must be taken not to smear the tissue on the slide, because this produces shearing forces, which can rupture cell and nuclear membranes.

CAUSES OF NONDIAGNOSTIC SPECIMENS

Cytology specimens of nondiagnostic quality may be the result of poor technique, but some are due to inherent problems with cytologic sampling. The latter include preparations that contain few cells because the tissue is difficult to sample; samples that consist of only blood because the lesion is vascular; samples in which the cells obtained are not necessarily representative of the lesion; tissue types in which it is difficult to distinguish normal from neoplastic; and samples in which the architecture of the tissue, rather than cell type, is critical for the diagnosis.

Problems related to poor technique include samples with low cellularity because the lesion was missed; samples that are too thick; preparations in which all the cells are broken; samples that consist only of blood because a too-large needle was used to collect the sample; slides with formalin fume artifact; samples that are over a week old when stained; slides from which the cells were removed when the slide was wiped to clean off the oil; and samples in which the cells are not representative of the lesion, often because an ulcerated tumor is imprinted rather than aspirated,. Cytology can be successful only if cells are present on the slide, are one cell layer thick, are not broken, and are adequately stained.

Low Cellularity

Slides may contain few or no nucleated cells because the target tissue was missed, the tissue was difficult to sample, or the sample was made up of fat cells that disappeared in the alcohol during the staining process. Obtaining cells from mesenchymal (connective) tissue is inherently more difficult. Some mesenchymal tumors may have relatively low cellularity because of the presence of a large amount of matrix, produced by the connective tissue, that surrounds the cells. The more benign the connective tissue tumor, the more matrix and the fewer cells it will have. Aspirates from lipomas usually contain very few cells, and what few cells are aspirated may wash off when the slide is dipped into alcohol, the first step in most of the rapid stain procedures. The practitioner should examine the slide before staining and make a note if the slide appears greasy or oily. Aspirated normal subcutaneous fat appears very similar to fat aspirated from a lipoma. Aspiration of perinodal fat is common during attempts to aspirate lymph nodes, especially popliteal lymph nodes.

Blood Contamination

Blood contamination commonly occurs if the needle used for aspiration is larger than 20 gauge. If platelets are present in the sample, the blood present can be assumed to represent contamination. Aspirates from some abdominal organs, such as the spleen and liver, tend to contain a large amount of blood. If macrophages are present that have phagocytized erythrocytes or that contain hemosiderin, a red blood cell breakdown product composed of iron, previous hemorrhage likely has occurred in the lesion. For example, hematomas usually have large numbers of macrophages that are filled with erythrocytes. Hemangiosarcomas, a type of mesenchymal tumor, are filled with blood and may yield few or no cells when aspirated. Although erythrocyte morphology usually is insignificant in cytologic preparations, an exception is the presence of acanthocytes in aspirates from hemangiosarcomas. This spiculated shape of red blood cells is very typical of patients with a hemangiosarcoma. As mentioned previously, with imprints of excised material, the imprint must be blotted with absorbent paper before the tissue is imprinted on the glass slide; otherwise, the imprint will consist of only blood or tissue fluid.

Cells Unrelated to the Primary Lesion

Inflammatory cells may surround a tumor, and sometimes only the inflammatory cells are aspirated. The needle should be redirected several times in lesions to

obtain cells from various parts; this is particularly important with enlarged lymph nodes in which metastatic neoplasia is suspected. Lesions that are usually diffuse, such as lymphoma, are easier to sample than are focal lesions, from which cells may not be obtained. A common example of obtaining cells that do not typify the primary lesion is cytologic preparations of nasal discharge. Typically only neutrophils are present, because nasal tumors do not tend to exfoliate cells into the discharge. The nasal mass itself usually must be aspirated or imprinted. If a cutaneous or subcutaneous mass is ulcerated, imprints of the ulcerated surface usually yield only neutrophils and bacteria rather than cells from the underlying mass. Attempts to aspirate submandibular lymph nodes may result in acquisition of only normal salivary gland epithelial cells, which should not be confused with cells that signal the presence of a carcinoma. Another example of the presence of confusing epithelial cells is putative thoracic aspirates that contain liver cells.

Misleading Cytologic Appearance of the Tissue

Transitional epithelial cells almost always appear malignant, even when normal, because they replicate rapidly and therefore exhibit some of the features considered criteria of malignancy. Rapidly dividing fibroblasts in an inflammatory lesion may have many of the features common to malignant cells. Mesothelial cells in body cavity effusions are easily mistaken for carcinoma cells. Epithelioid macrophages in inflammatory lesions can appear very similar to epithelial cells. Nasal carcinomas, on the other hand, often appear quite benign, because the cells often are small and uniform. Neoplasms of endocrine tissue, such as thyroid or perirectal apocrine gland adenocarcinomas (which behave as do endocrine tumors, producing parathyroid hormone–related peptide), also often appear quite benign, even though their behavior usually is malignant.[1] Osteoblasts from well-differentiated osteosarcomas may appear similar to osteoblasts from normal or reactive bone.

Too-Thick Preparation

Cytology is successful only when cells are one layer thick and unbroken. Some tissues, such as lymph nodes, bone marrow, and some tumors, yield many cells, and the preparation therefore may be inherently thick. In these situations, one usually can find areas thin enough to examine at the edge of the smear. Too-thick preparations also are seen with "squirt" preparations, in which the cells are squirted onto the slide from the syringe and not spread thinly across the slide.

Inadequately Stained Sample

Inadequate staining may simply be a result of not dipping the sample long enough in the stain or of not changing the stain often enough. Stain should be changed weekly, not only because it eventually stains less intensely, but also because many of the quick Wright-Giemsa–type stains (e.g., Diff-Quik and Quick-Dip) support the growth of bacteria and fungi. These contaminants may be mistaken for pathogens when the preparation is examined. Other reasons for inadequate staining include samples that are too thick; contamination of the sample with formalin fumes; and not staining the sample within 1 week of collection. Formalin fume contamination is a common problem, because samples often are prepared next to the formalin jar, or samples are shipped in the same box as bottles containing formalin. Cells that have been fixed by formalin fumes do not take up the stain and appear as light blue structures without any nuclear detail. Stain precipitate usually is not a problem when dip–type stains are used, but it sometimes can form in aged stain. Stain precipitate usually appears as clumps of purple dots and may be mistaken for bacteria.

Cells Too Degenerated (Broken) to Identify

Degeneration of cells may be unavoidable when necrotic tissue is aspirated. More commonly, cells are broken during the preparation process. Certain types of cells, such as lymphoblasts, are quite fragile. Cells must be distributed on the slide quite gently; as described previously, this is done by placing one slide over the slide with the aspirated cells, allowing the material to spread out, then pulling the slides apart in a gentle manner. If these types of preparations are made as blood films are, by using a push-type technique, cells often break. Cells also break if they are incorporated into clots. Aspirated material should be placed on a slide and spread out within approximately 30 seconds of collection. If this cannot be accomplished, a few drops of ethylenediamine tetra-acetic acid (EDTA) from a purple-top Vacutainer tube can be added to the syringe into which the cytology sample has been collected.

Broken nuclear material usually streams across the microscope slide and may be mistaken for fibrin or spindle-shaped cells. Another cause of cell degeneration is the environment to which the cells are exposed. For example, cells from airway washings come in contact with saline and often are degenerate, as are cells in urine. Aging of a sample before slide preparation results in degeneration of cells. If the aspirated material is a fluid type, films should be made within 30 minutes of collection to prevent degeneration of cells.

Cells can be broken or even removed after staining and examination of a slide if an attempt is made to wipe the immersion oil from the slide. Instead, a solvent such as Americlear or Safe Clear should be used to remove oil.

Broken Slides

Samples to be mailed to a laboratory should be wrapped carefully in bubble wrap or put in hard plastic boxes. The small cardboard folder mailers provide inadequate protection and do not prevent the slides from being broken in transit.

Poor-Quality Microscope

Inexpensive microscopes often are of inadequate quality to allow proper examination of cytologic specimens. Objectives should be planachromatic, allowing the entire field to be in focus. Coverslips should be placed on slides when the 40× objective is used because they sharpen the image. To obviate the need for coverslips, 50× oil objectives can be used. The most common reason for failure to focus is placing the slide on the microscope with the wrong side up. Oil should be removed from the oil objective after each use. Failure to remove oil can result in permanent lens damage.

Nondiagnostic specimens cannot always be avoided; however, if proper technique is used, the percentage of nondiagnostic specimens declines significantly.

GENERAL APPROACH TO EXAMINATION OF THE MICROSCOPE SLIDE

Preparations should be examined initially with a low-power objective. Most ocular lenses magnify by 10, and when used with a 10× objective lens, the cells are magnified 100-fold. At this magnification, the examiner can evaluate cell density and observe the different cell types present, and thereby recognize multiple processes. For example, both neoplastic and inflammatory cells may be present on the same slide. Perhaps most important, while studying the sample at low power, the examiner can find a suitable area to examine at higher magnification. Unlike blood films, cytologic preparations do not have one area to examine that is preferable over another. The preparation must be scanned so that no diagnostic cells are missed and also to detect an area that is not too thick, is stained adequately, and contains intact cells.

When a slide is examined with the oil immersion objective lens (50× or 100×), cells are magnified 500- or 1000-fold. At this magnification, various cell types can be identified; cellular detail (e.g., the presence of nucleoli) can be examined; and the sample can be searched for small microorganisms. The examination is followed by a complete description and interpretation of the preparation. The description should include information about the degree of cellularity, the predominant cell type, and the morphologic appearance of various cells and other structures, including microorganisms. An interpretation of these findings is then provided. Interpretations are discussed in more detail later in the chapter. Briefly, they usually include whether the preparation is indicative of inflammation (including the type and etiology, if discernible) or of neoplasia, and if the latter, the type of neoplasia.

CELL TYPES COMMONLY SEEN IN CYTOLOGIC SPECIMENS

Erythrocytes

Red blood cells are similar in appearance to those seen on peripheral blood films, although the erythrocyte morphology may be altered. Some erythrocytes are seen in most tissue aspirates as a result of capillary damage that occurs during acquisition of the samples. Hemorrhage in a tissue can be diagnosed by the presence of macrophages that have phagocytized erythrocytes or that contain hemosiderin, an erythrocyte breakdown product that contains iron. Hematoidin crystals, a golden crystalline erythrocyte breakdown product, may also be observed in hemorrhagic lesions. Platelets may be present in samples that are contaminated with fresh blood.

Inflammatory Cells

In general, the more inflammatory cells that are present, the less likely it is that the lesion is neoplastic. A common exception to this is squamous cell carcinoma, which typically elicits an inflammatory reaction. Neutrophils are present in very low concentration in many non-inflammatory lesions and in high concentration in inflammatory lesions. Neutrophils usually are well preserved (nondegenerate) in nonseptic inflammatory lesions, appearing much as they do in peripheral blood. As the neutrophils age, the nuclei become hypersegmented and eventually pyknotic. In septic inflammatory lesions, the neutrophils undergo rapid degeneration and eventual rupture. This lytic process, called *karyolysis*, is characterized by nuclear swelling, which gives the nucleus a pink-stained and smudged appearance. The cytoplasm usually becomes more basophilic and vacuolated, but its appearance is relatively unimportant in determining whether cells are degenerate. When karyolysis is observed, thinly smeared areas should be carefully examined for bacteria within the cytoplasm of

the neutrophils. Bacteria, all of which stain blue with Romanowsky (Wright's-type) stains, are discrete, uniform structures that should not be confused with mast cell granules, background protein material, or stain precipitate. Background protein is the fine, eosinophilic granular material seen between cells in high-protein fluids. Precipitated stain, resulting from inadequate rinsing of the slide, is extremely variable in size and shape, is usually dark purple, and may resemble chains of cocci.

Lymphocytes usually are present in low concentration in most cytologic material and in high concentration from lymphoid tissue and lesions caused by lymphatic rupture or lymphoma. Normal lymphocytes appear similar to those in peripheral blood. Immature lymphoid cells (lymphoblasts) are characterized by their large size and the presence of a nucleolus. Mature lymphoid cells also have a nucleolus, but it is masked by nuclear chromatin; when the nuclear membrane of these cells is ruptured, the nucleolus often becomes apparent. When lymphocytes are antigenically stimulated, they may transform into large cells with a more abundant, more basophilic cytoplasm. They may also undergo blast transformation to become plasma cells.

Plasma cells are most commonly seen in chronic inflammatory lesions. They indicate antibody production as a result of antigenic stimulation. They are similar in size to small lymphocytes, but the nuclear chromatin is more dense, the cytoplasm is blue and abundant, and a perinuclear clear area (Golgi apparatus) usually is apparent.

Macrophages in tissues and body fluids are derived from blood monocytes. These cells phagocytize cellular debris, foreign material, and certain microorganisms, primarily mycobacteria. Other bacteria are not commonly seen in the phagocytic vacuoles of macrophages. Neutrophils, cellular debris, erythrocytes, red blood cell pigments, lipids, and phospholipids are frequently seen in the cytoplasm of these phagocytic cells. Macrophages range in size from 12 to 100 μm, have a round to oval nucleus that may have an apparent nucleolus, and have a light blue, usually vacuolated cytoplasm. Macrophages may become multinucleated (up to 16 or more nuclei may be observed) and are then sometimes referred to as *giant cells.* If a macrophage is not actively phagocytic, it may be similar in appearance to an epithelial cell, and it is then classified as an epithelioid macrophage; care must be taken not to misidentify epithelioid macrophages as carcinoma cells.

Eosinophils in cytologic preparations appear similar to eosinophils seen on blood films. When present in material containing a large amount of mucus, such as tracheal washings, the granules may stain brown rather than the characteristic bright pink. Eosinophils are present in lesions in which allergic responses occur and are usually present in large numbers in aspirates of mast cell tumors.

Mast cells are present in low concentration in many tissues. They are round cells with round to oval nuclei and cytoplasm that contains purple granules. They are almost always the predominant cell type in aspirates of mast cell tumors.

Noninflammatory, Nonneoplastic Cells

Epithelial Cells

Epithelial cells are seen in aspirates from epithelial tissue, such as vaginal and oral mucosa, and aspirates of skin and glandular tissue, such as salivary and mammary glands. Epithelial cells are quite variable in size and have cell-to-cell association, or bridging. Nonglandular epithelial cells tend to be round with a moderate amount of pale to medium blue cytoplasm. They have a single round to oval, purple-staining nucleus with smooth chromatin and often contain a small, round nucleolus. Pigmented epithelium contains melanin granules that are gray to black. As squamous epithelium matures, cells develop the morphology of typical superficial squamous epithelial cells, which are quite large, appear flat, have abundant cytoplasm that stains light blue to blue-green, and have a small, contracted, dark-staining (pyknotic) nucleus or are anucleate. Glandular (exocrine) epithelial cells usually have abundant, foamy-appearing cytoplasm. Endocrine gland epithelial cells (e.g., thyroid and parathyroid) have less cytoplasm that usually is not vacuolated.

Mesenchymal Cells (Connective Tissue Cells)

Cells of connective tissue origin tend to be spindle or fusiform, with cytoplasmic tails trailing away from the nucleus. These cells do not exfoliate as easily as epithelial cells. Mesenchymal cells originate from any of the connective tissues in the body, such as fibrous connective tissue, cartilage, and bone. Connective tissue cells (fibroblasts) are commonly seen in aspirates from pyogranulomatous inflammatory lesions. Fat cells (lipocytes, adipocytes) may be aspirated, particularly from bone marrow and lymph nodes. These cells, unlike other connective tissue cells, are very large (approximately 50 to 100 μm); they have clear cytoplasm, representative of the location of large fat globules and a distinct, sometimes folded cytoplasmic membrane. The nucleus is small and dense and usually pressed against the cytoplasmic membrane.

INTERPRETATION OF THE SPECIMEN

As mentioned previously, a general approach to interpretation of cytologic findings involves first determining whether the specimen is neoplastic or inflammatory and then the type of neoplasia or inflammation present. The diagnosis of neoplasia is based on the presence of
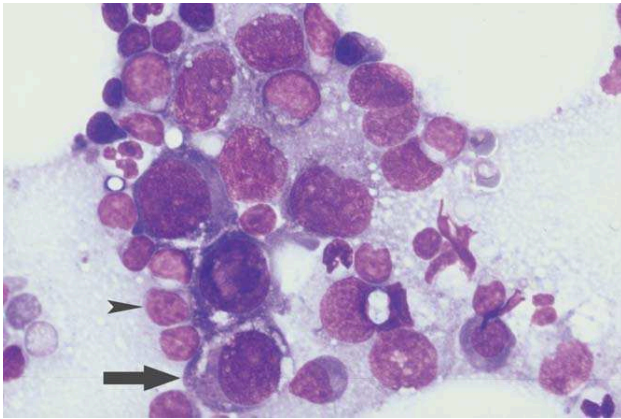
Figure 7-1

Lymph node aspirate from a dog with metastatic carcinoma. Epithelial cells *(arrow)* are present, as are small, normal-appearing lymphocytes *(arrowhead)*.

Figure 7-2

Carcinoma cells. Numerous features of malignancy can be seen, including nuclear molding, variability in cell size, and multinucleated cells with variable-size nuclei.

cells with malignant characteristics or of cells foreign to the tissue source. For example, epithelial cells should not be found in lymph node aspirates (Figure 7-1). If the cell morphology suggests that the specimen is neoplastic, the tumor usually can be classified as a discrete (round) cell tumor, a tumor of epithelial origin, or a tumor of connective tissue origin. Neoplasms, especially squamous cell carcinoma, may induce an inflammatory response; as a result, the cytologic findings may be interpreted as both inflammatory and neoplastic.

Cellular Criteria of Malignancy

No single criterion denotes neoplasia, nor do all tumor cells exhibit all criteria. Some criteria do not apply to certain tumor types. As a general rule, several criteria of malignancy should be present before a diagnosis of neoplasia is considered.

Nuclear Criteria

Nuclear changes in malignant cells reflect increased nuclear activity or replication. Some manifestations of nuclear criteria are found in cells undergoing *hyperplasia*, or controlled growth (e.g., fibroblasts in granulation tissue). Common nuclear criteria include variable nuclear size (anisokaryosis); a variable and usually increased nucleus-to-cytoplasm ratio; abnormally clumped chromatin patterns; and large, multiple, irregularly shaped nucleoli. Other nuclear changes sometimes observed include abnormal mitoses and nuclear molding, which is the presence of nuclei in multinucleated cells in which one nucleus conforms to the shape of another nucleus (Figure 7-2). Although multinucleation and normal-appearing mitotic figures are commonly observed in neoplastic cells, they also are observed in normal cells such as fibroblasts, macrophages, and mesothelial cells.
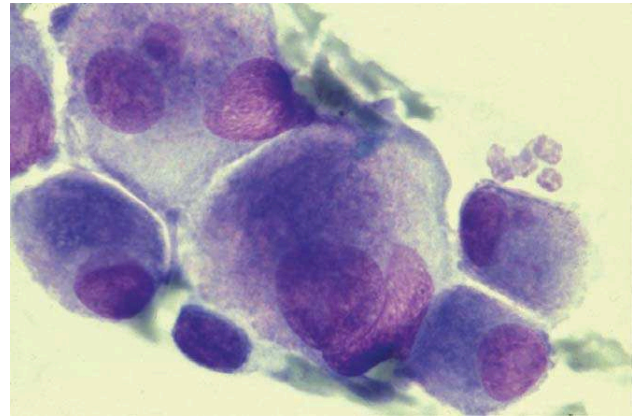
Cytoplasmic Criteria

Cytoplasmic criteria of malignancy, which are less important than nuclear criteria, include increased basophilia and vacuolation. Both of these features are more common in tumors of epithelial origin. Vacuoles in tumor cells sometimes simply reflect rapid growth and degeneration. In adenocarcinoma cells, however, characteristic perinuclear vacuolation, representative of the secretory and packaging role of the cell, is commonly observed. When large secretory droplets compress the nucleus to the side of the cell, the cell is known as a *signet ring cell*. Normal cells that imbibe fluids can also have this appearance. Neoplastic cells sometimes are phagocytic or cannibalistic and will phagocytize erythrocytes or other tumor cells.

Structural Criteria

Certain types of neoplastic cells become very large. Neoplastic epithelial cells sometimes replicate without dividing, resulting in long chains of attached cells. Epithelial cells have intercellular connections, and large clumps of connected cells often can be observed. Acini can sometimes be seen in aspirates from neoplastic glandular epithelium. Cellular crowding (piling) is a common feature of aspirates from malignant epithelial tissue.

CLASSIFICATION OF NEOPLASMS

Discrete Round Cell Tumors

Cells from round cell tumors are small, discrete (individualized), and round. Lymphoma, plasma cell tumors, histiocytomas, mast cell tumors, transmissible venereal tumors, and malignant histiocytosis are typically classified as discrete cell tumors.
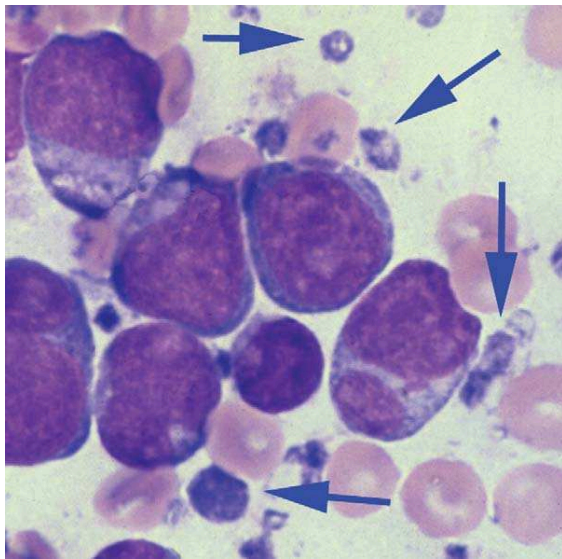
Figure 7-3

Aspirate from a lymph node in a dog with lymphoma. Numerous lymphoblasts are present. Cytoplasmic fragments are indicated by arrows.

Lymphoma

Lymphoma can be found in virtually any organ.[2-4] Neoplastic lymphoid tissue may consist of either small lymphocytes or large lymphoblasts, but the cells more often are lymphoblastic. When lymphoblastic cells are present, the cytologic diagnosis often is relatively simple. Lymphoblasts are the predominant cell type and are approximately twice the size of neutrophils. The cytoplasm of lymphoblasts is scant, light to medium blue, and sometimes vacuolated. The nuclei usually are indented, have fine to stippled chromatin, and generally contain one to three prominent nucleoli (Figure 7-3). Types of lymphoblastic lymphoma can be further subdivided according to cellular morphology, histopathologic appearance, and whether the lymphoblasts originate from B cells or T cells. The diagnosis of lymphoma in tissues other than lymph nodes usually is quite simple, because lymphoblasts should not be present in large numbers in the skin, bone marrow, liver, intestine, or other nonlymphoid organs. Lymphoblasts are fragile and frequently are broken during preparation. Cytoplasmic fragments, sometimes referred to as *lymphoglandular bodies*, often are abundant.

If neoplastic lymphoid cells are small, they usually have abnormal features, such as retained nucleoli or increased cytoplasm, often with projections, or *pseudopodia* (Figure 7-4). Polymerase chain reaction (PCR) techniques or histopathologic examination may be required to confirm the diagnosis of small cell lymphoma.[5]

Because clonality is the hallmark of malignancy, an assay has been developed that uses PCR to amplify
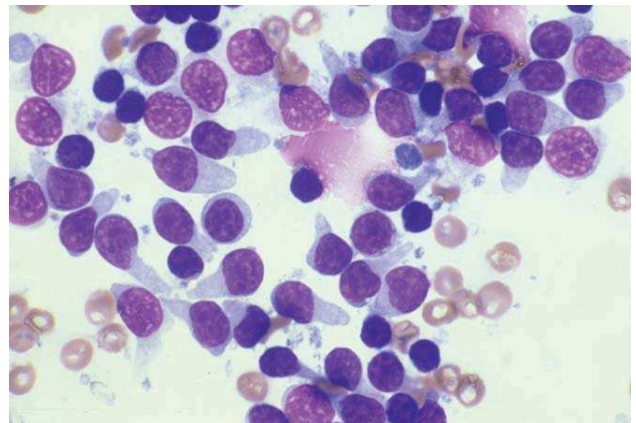
Figure 7-4

Aspirate from a lymph node from a dog with a small cell variant of lymphoma. The neoplastic lymphocytes are slightly larger than normal and have abundant cytoplasm with cytoplasmic projections.

the variable regions of immunoglobulin genes and T-cell receptor (TCR) genes to detect the presence of a clonal lymphocyte population. Gene rearrangement is appropriate for the immunophenotype (immunoglobulin gene rearrangement in B-cell lymphoma, and TCR gene rearrangement in T-cell lymphoma). The clonal rearrangement can be detected when very small amounts of the deoxyribonucleic acid (DNA) are derived from neoplastic cells. Because all lymphomas are clonal expansions of lymphocytes, each particular neoplasm has DNA regions that are unique in both length and sequence. The CDR3 region of both immunoglobulin and TCR genes encodes the antigen-binding region of the respective receptor and contains the majority of this unique sequence.

Although it is assumed that malignancy is always clonal, all clonal expansions of lymphocytes are not necessarily malignancies; it is important to consider this possibility if samples have clonal rearrangements but no other evidence of lymphoma. Aspirates of lymph nodes (or other organs) can be submitted in approximately 1 ml of physiologic saline. Two or three aspirates (with rinsing of the syringe in the saline each time) provide adequate material. Alternatively, stained or unstained cytologic preparations can be scraped and PCR performed on the scraped cells. (Samples may be sent to the Colorado State University Diagnostic Laboratory, Fort Collins, CO 80523, Attention: Dr. Anne Avery.)

Plasma Cell Neoplasia (See Chapter 31 Section D)

Plasmacytomas are solitary plasma cell tumors that may develop subcutaneously and in the oral cavity. These tumors consist of plasma cells that may be well differentiated, somewhat immature, or poorly differentiated.
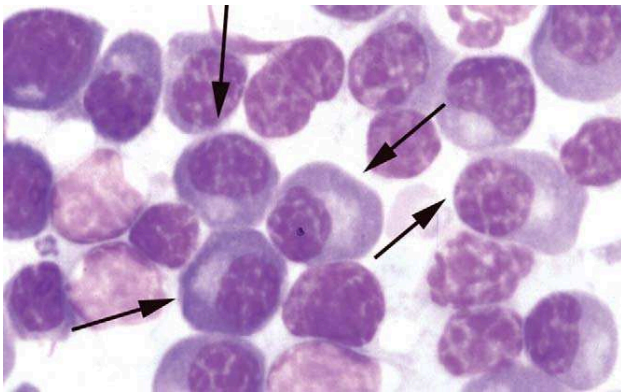
Figure 7-5

Aspirate from a plasmacytoma. Note the numerous plasma cells *(arrows)* with eccentric nuclei and clear Golgi areas in the cytoplasm.

Figure 7-6

Bone marrow aspirate from a dog with multiple myeloma. Almost all the cells present are plasma cells *(arrowheads)*. The arrow indicates a megakaryocyte.

They have small nuclei with stippled chromatin, abundant basophilic cytoplasm, and often a clear perinuclear Golgi area (Figure 7-5). Numerous binucleated or trinucleated plasma cells often are present. Poorly differentiated plasma cell tumors may appear similar to malignant histiocytosis cells or amelanotic melanoma cells. Plasma cell myeloma (multiple myeloma), on the other hand, is a relatively rare lymphoproliferative neoplasm in which plasma cells or their precursors proliferate abnormally. As implied by the term *multiple myeloma*, neoplastic plasma cells proliferate in the bone marrow at multiple sites and also are commonly present in the spleen and liver; they are rarely seen in the lymph nodes. A markedly increased concentration of plasma cells in the bone marrow (more than 20% of all nucleated cells) usually is the result of plasma cell neoplasia; however,

Figure 7-7

Aspirate from a histiocytoma on the pinna of a 1-year-old dog. The cytoplasm of the histiocytoma cells typically appears lighter than the background proteinaceous material.

plasma cell proliferation may also occur secondary to chronic antigenic stimulation.

Neoplastic plasma cells in the bone marrow often are seen in large aggregates. They sometimes appear slightly abnormal or immature, and some multinucleated plasma cells may be present. However, neoplastic plasma cells may also appear very well differentiated, making them difficult to distinguish from normal plasma cells (Figure 7-6). An important diagnostic and clinical manifestation of plasma cell myeloma is the presence of a monoclonal or biclonal gammopathy, usually IgG or IgA but occasionally IgM.

Histiocytomas (See Chapter 32, Section F)

Although histiocytomas typically are considered tumors of young dogs, they also are seen in middle-aged and old dogs. They are benign in behavior and appearance and usually regress spontaneously. The cells have a moderate amount of pale blue cytoplasm and round nuclei with fine chromatin and indistinct nucleoli (Figure 7-7). As the tumors regress, they are infiltrated with small lymphocytes, which may be present in higher concentration than the histiocytoma cells. The background fluid in aspirates often is darker than the cytoplasm of the cells, giving the cytoplasm a pale appearance.

Mast Cell Tumors (See Chapter 19)

Mast cell tumors consist predominantly of mast cells, which are round cells that usually are slightly larger than lymphoblasts. They have variable numbers of distinctive small, purple-staining granules in the cytoplasm that may obscure the nucleus (Figure 7-8). The nucleus is round to oval and usually stains somewhat pale, presumably because the granules take up the stain. The cells are somewhat fragile, and free granules usually
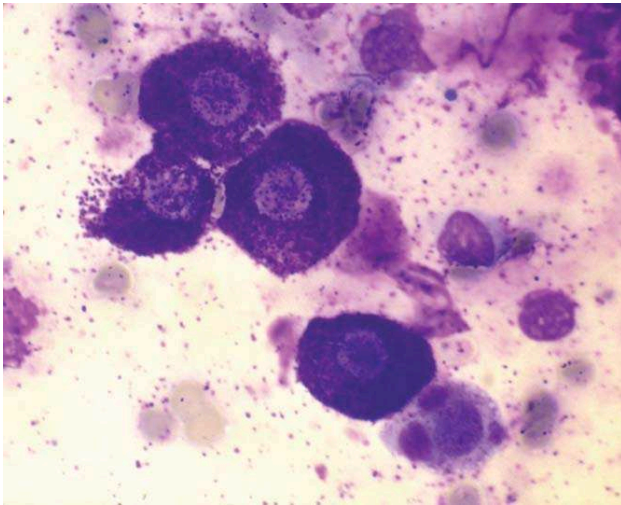
Figure 7-8

Aspirate from a mast cell tumor in a dog. Note the free mast cell granules in the background.
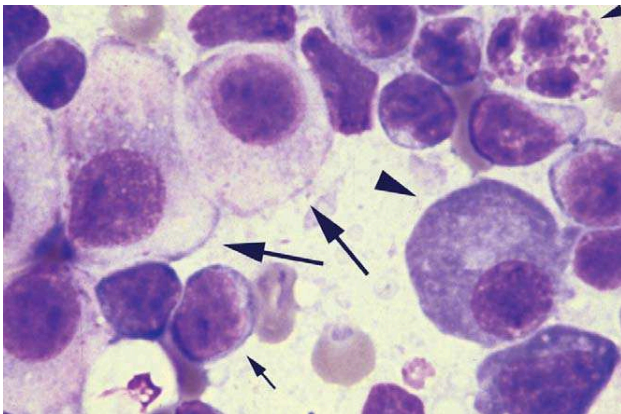
Figure 7-9

Aspirate from a lymph node with metastatic mast cell neoplasia, stained with a quick Wright's stain. The granules in the mast cells are poorly stained *(large arrows)*. Small lymphocytes are indicated by the small arrow. The arrowhead indicates a plasma cell.

are present. Eosinophils often are present in aspirates from mast cell tumors. Some mast cell tumors have large numbers of mesenchymal cells scattered throughout the aspirate, and pink, amorphous material, probably degenerated collagen, may also be seen. The granules in a small percentage of mast cell tumors do not stain with some of the quick stains, such as Diff-Quik (Figure 7-9). Mast cell neoplasms diagnosed by cytology should be widely excised, submitted for histopathologic examination, and graded to help determine a prognosis. A cytologic grading system with prognostic significance has not yet been developed.
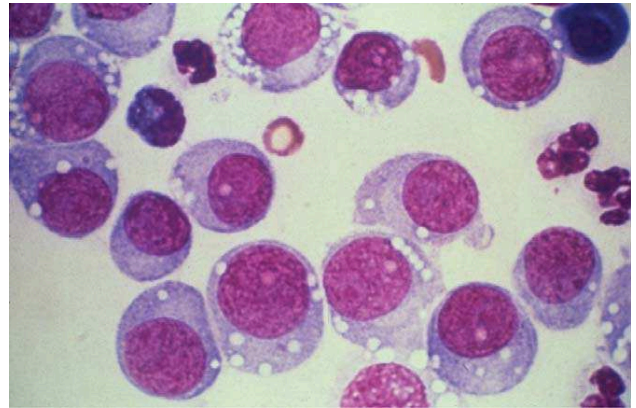
Figure 7-10

Aspirate from a transmissible venereal tumor. Note the abundant cytoplasm with clear distinct vacuoles. *(Courtesy Dr. Robert Hall.)*

Transmissible Venereal Tumors (See Chapter 33C)

Transmissible venereal tumors (TVTs) are sexually transmitted tumors of dogs. They often are malignant in behavior but respond well to chemotherapy and radiation therapy. They consist of discrete cells with a moderate amount of light blue cytoplasm that sometimes is filled with numerous small, distinct, clear vacuoles (Figure 7-10). The nuclei are round and have coarse chromatin and large, prominent nucleoli. TVTs usually are found on mucous membranes, such as the penis, vagina, and nose, but they can metastasize to many other areas.

Malignant Histiocytosis (See Chapter 32, Section F)

Malignant histiocytosis (systemic histiocytic sarcoma) is a rapidly progressive, ultimately fatal, proliferative disorder of the mononuclear phagocyte system. It has been described in adult dogs, including Bernese mountain dogs and other breeds; it is relatively rare in cats. A higher incidence of the disorder has been suggested in the golden retriever and flat-coated retriever breeds.[6] Malignant histiocytosis often is characterized by the systemic proliferation of large, pleomorphic, single-nucleus and multinucleated histiocytes with marked cellular atypia and phagocytosis of erythrocytes and leukocytes (Figures 7-11 and 7-12). The marrow commonly is involved, as is the lung, lymph nodes, liver, spleen, and central nervous system. Positive reactivity of neoplastic cells to histiocytic markers (e.g., lysozyme and alpha-1-antitrypsin) and more advanced immunophenotypic markers can be demonstrated by immunohistochemistry, which aids differentiation of neoplastic histiocytic cells from lymphoid and epithelial neoplasms and
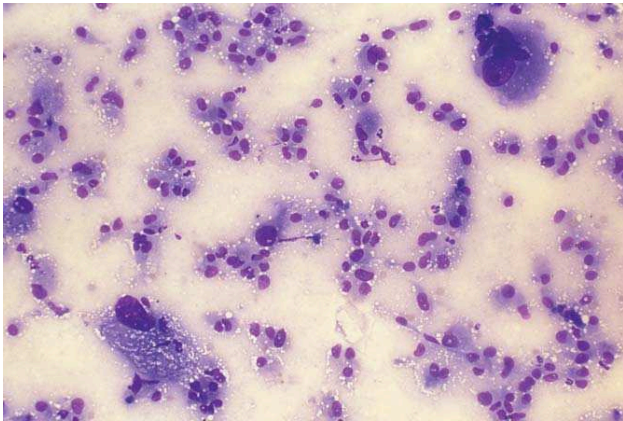
Figure 7-11

Splenic aspirate from a dog with malignant histiocytosis (low magnification). Almost all the cells present are malignant histiocytoma cells. Note the variability in cell size.
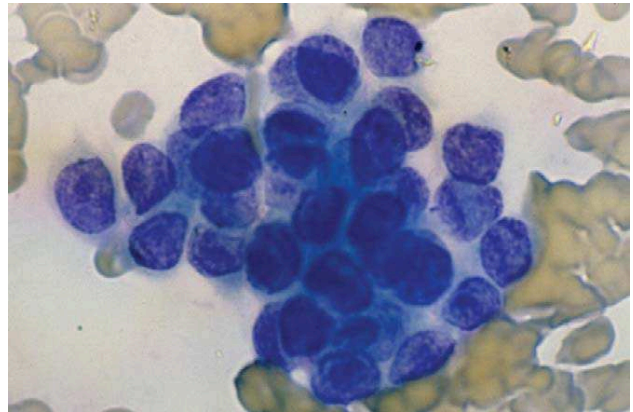
Figure 7-13

A cluster of basal epithelial cells from a basal cell tumor aspirate. Note the uniform size of the nuclei and the absence of features of malignancy.
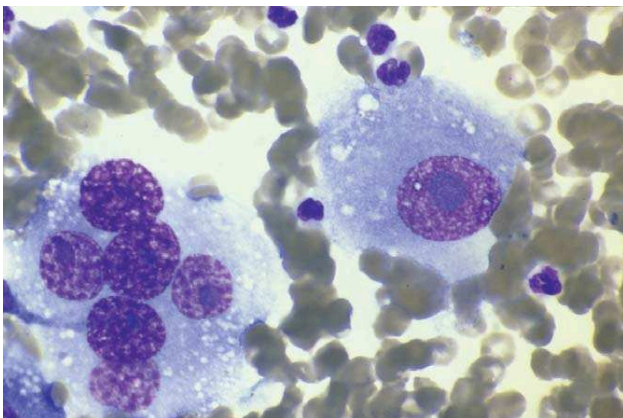
Figure 7-12

Aspirate from a lymph node in a dog with malignant histiocytosis. Note the multinucleated cell on the left, and the very large nucleolus in the cell on the right.

is helpful for making the definitive diagnosis of the neoplasm. Neoplastic histiocytes are pleomorphic, large, discrete, markedly atypical mononuclear cells with nuclei that are round to oval or reniform and abundant, lightly basophilic, often vacuolated cytoplasm.

Features of malignancy include marked anisocytosis and anisokaryosis; prominent nucleoli, and bizarre mitotic figures. The presence of multinucleated giant cells also supports the diagnosis. The cells are thought to arise from dendritic antigen-presenting cells. Aspirates from malignant histiocytosis tumors can appear similar to those from malignant fibrous histiocytomas; they usually are quite easy to differentiate from aspirates of granulomatous inflammatory lesions.

Tumors of Epithelial Origin

Neoplastic epithelial cells usually are much larger and have more cytoplasm than discrete tumor cells, and they typically exhibit many of the features of malignancy discussed previously. Because epithelial cells tend to adhere to each other, clusters or clumps of cells usually are seen in aspirates. Cell-to-cell relationships usually are apparent. The nuclear chromatin may be fine to coarse, and nuclei have one or more nucleoli that may be quite large and prominent when cells are malignant. Nuclear molding may be present in multinucleated cells. Various types of epithelial tumors, such as thyroid carcinomas, basal cell tumors, perianal gland adenomas, squamous cell carcinomas, and sebaceous cell adenomas, have specific characteristics that aid identification.

Basal Cell Tumors (See Chapter 18)

Basal cell tumors are relatively common in dogs and cats. They are similar to other epithelial cell tumors, but the cells are small and quite uniform. Basal epithelial cells may be mistaken for those of a round cell tumor, because they may appear as individual cells with a high nucleus-to-cytoplasm ratio. However, basal epithelial cells typically appear in rows and clusters. The nucleus is small and has a somewhat dense chromatin and a small, indistinct nucleolus (Figure 7-13). The cytoplasm is scant and lightly basophilic to gray. Basal epithelial cells may be pigmented (contain melanin) and can be confused with cells from melanomas, although malignant melanoma cells almost always exhibit more features of malignancy. Some basal cells may be seen that are differentiating into sebaceous cells (see the following section).
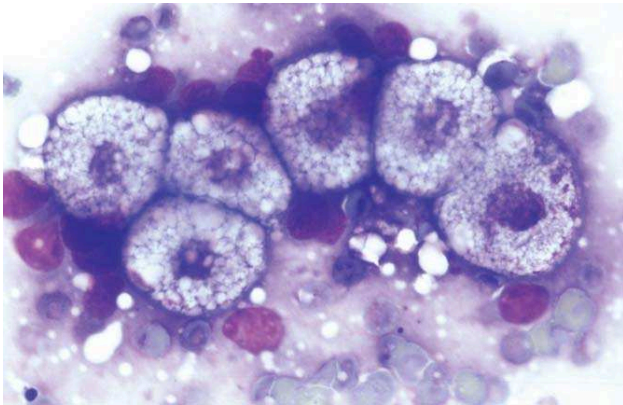
Figure 7-14

Aspirate from a sebaceous cell adenoma. Note the numerous, uniform vacuoles in the cytoplasm of the cells.

Figure 7-15

Aspirate from a squamous cell carcinoma (low magnification). Note the numerous epithelial cells with abundant cytoplasm. The arrow indicates a cell in mitosis.

Sebaceous Cell Tumors (See Chapter 18)

Sebaceous cell adenomas are the most common benign skin tumor in dogs, especially cocker spaniels and poodles. They also occur in cats. These neoplasms often develop on the head and neck, and they may have a wartlike appearance on gross examination. Sebaceous cell tumors are characterized by the presence of cells with numerous small, relatively uniform, clear vacuoles in the abundant cytoplasm (Figure 7-14). The nucleus usually is small, round, and dark. These cells can be distinguished from macrophages by the lack of phagocytic activity and the uniform size and shape of the vacuoles. Basal epithelial cells may also be present.

Sebaceous cell carcinomas are uncommon. Cells from these tumors show features of malignancy, including variability in nucleus and cell size and large nucleoli. They sometimes may be confused with liposarcoma cells because of the numerous clear vacuoles in the cytoplasm. The two cell types generally can be distinguished by the fact that carcinoma cells usually are found in groups, and they adhere to each other.

Squamous Cell Carcinomas (See Chapter 18)

Squamous cell carcinomas are common tumors of older domestic animals. They may arise on the skin, oral cavity, stomach, respiratory tract, and reproductive tract. These tumors usually can be identified by the presence of very large, superficial squamous epithelial cells, which often are keratinized and have retained nuclei. Epithelial cells show features of malignancy, including the presence of nuclei and large nucleoli (Figure 7-15). Well-differentiated squamous cell carcinomas may be difficult to differentiate from benign lesions that have superficial epithelial cells and keratin, such as epithelial inclusion cysts and cornifying epitheliomas. However, cysts and epitheliomas do not have epithelial cells with large nuclei or nucleoli. Aspirates from squamous cell carcinomas often contain inflammatory cells, possibly because the keratin elicits an inflammatory reaction.
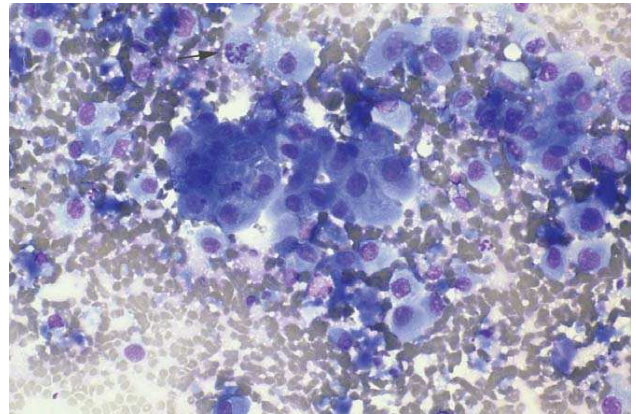
Perianal Gland Tumors (See Chapter 21, Section H)

Cells from perianal gland adenomas and adenocarcinomas have a very characteristic hepatoid appearance because the cells closely resemble hepatocytes. The cytoplasm is abundant and light blue; the nuclei are centrally located, round, and uniform and usually have one distinct nucleolus. Basal epithelial cells may be present. Carcinomas are difficult to distinguish from adenomas based on cytology. However, if a large amount of cellular variability is present and the nucleus-to-cytoplasm ratio is smaller than usual, a carcinoma should be suspected.

Apocrine Gland Adenocarcinomas of the Anal Sacs (See Chapter 21, Section H)

Cells from perirectal apocrine gland adenocarcinomas exfoliate easily, but the cells usually are broken. Consequently, many nuclei are present with no visible cytoplasm. Typically, some cells are in clusters, indicating that the cells are epithelial in origin. Intact cells have a small nucleus-to-cytoplasm ratio, and the small amount of cytoplasm usually is light blue. The nuclei are uniform and round to oval; the chromatin pattern usually is fine, and small nucleoli usually are present (Figure 7-16). Although the cells appear relatively benign on cytology, their behavior is very malignant, and many of these tumors have metastasized by the time of initial diagnosis. Many of these patients are hypercalcemic, because the tumors produce parathyroid hormone–related peptide (PTH-rp).
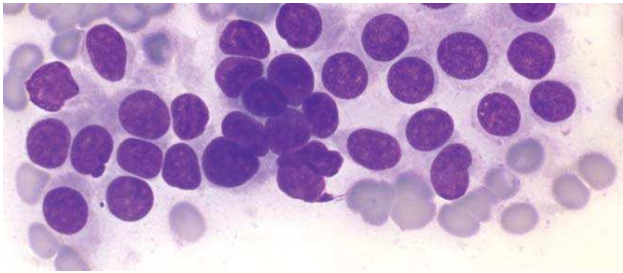
Figure 7-16

Aspirate from an apocrine gland adenocarcinoma of the anal sac in a female dog. Note the uniformity and high nucleus-to-cytoplasm ratio of the cells.
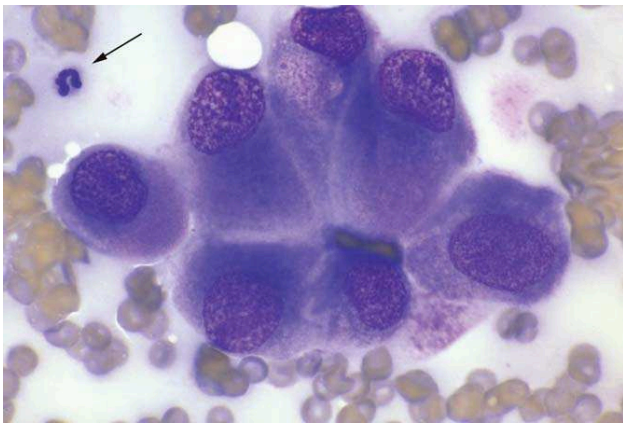
Figure 7-17

Mammary gland carcinoma. Note the large size of the cells compared to the neutrophil *(arrow)*. The cells have large, multiple nucleoli and are arranged in an acinar formation.

Mammary Gland Tumors (See Chapter 26)

Aspirates of mammary gland tumors are extremely variable in appearance. Neoplasia usually can be differentiated from inflammation or hyperplasia. However, benign and malignant tumors can be difficult to differentiate unless cellular features of malignancy are obvious.[7,8] As with other carcinomas, features of malignancy include cellular piling, variability in cell size and shape and in nuclear size, and large, prominent nucleoli (Figure 7-17). Adenocarcinoma cells may have a secretory product in the cytoplasm.

Thyroid Gland Tumors (See Chapter 24)

Thyroid gland tumors usually are found in the midventral cervical region. Aspirates of these tumors are similar to those of other endocrine tissue.[1] They usually have few to a moderate number of relatively small, uniform-appearing epithelial cells, many of which are broken (Figure 7-18). Numerous free nuclei usually are present,
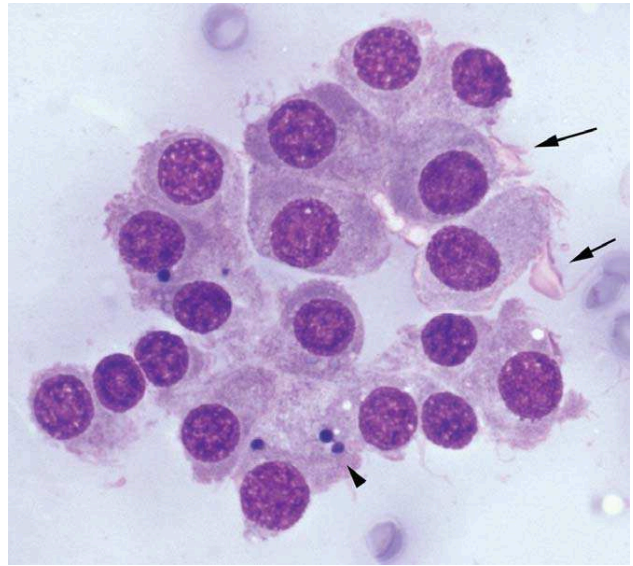
Figure 7-18

Aspirate from a thyroid gland carcinoma in a dog. Note the uniformity of the cells, the thyroglobulin *(arrows)*, and the pigment in the cytoplasm *(arrowhead)*.
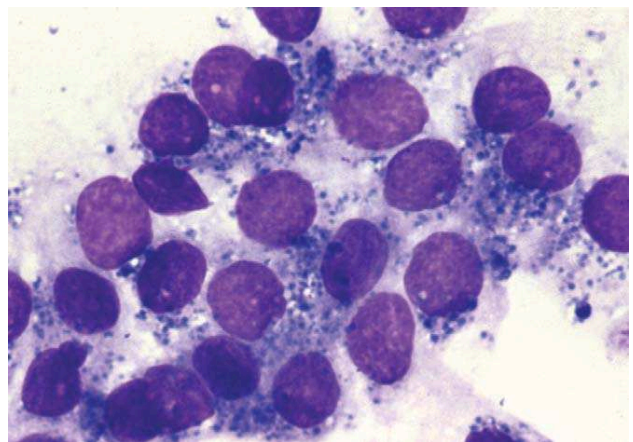
Figure 7-19

Aspirate from a thyroid gland carcinoma in a dog. Note the large number of cells that contain pigment.

and the aspirate often has many erythrocytes. The cytoplasm of some cells may contain blue-black granules, thought to be tyrosine (Figure 7-19). The nuclei usually are centrally located and have clumped chromatin. Features of malignancy often are not present, even in carcinomas; this makes the rare adenomas difficult to distinguish from carcinomas. Tumors with more aggressive cytologic features are characterized by some of the above observations, along with moderate to marked cellular and nuclear variability, multinucleation, a high
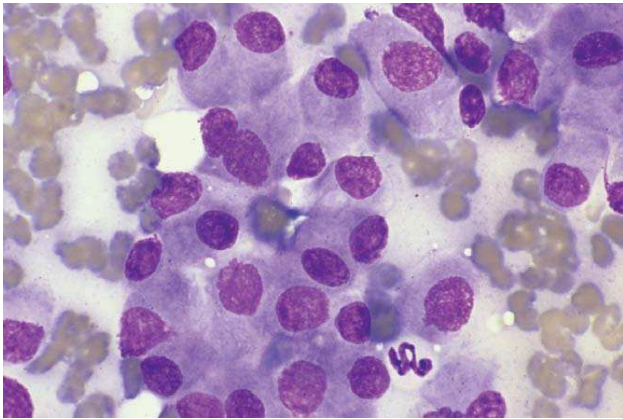
Figure 7-20

Aspirate from a parathyroid gland tumor in a dog that had hypercalcemia and hypophosphatemia. Note the uniformity of the cells.

Figure 7-21

Very high magnification of a binucleate sarcoma cell from a splenic hemangiosarcoma. Note the attenuated cytoplasm with tail-like points.

nucleus-to-cytoplasm ratio, and cellular piling. Most thyroid tumors in dogs are carcinomas, and almost all thyroid tumors in cats are adenomas. Parathyroid gland tumors (Figure 7-20) and C-cell tumors of the thyroid gland are similar in appearance to thyroid gland tumors.

Neuroendocrine Tumors

Neuroendocrine tumors are similar in appearance to thyroid tumors. They typically show large numbers of broken cells, similar to thyroid tumors and apocrine gland adenocarcinomas of anal sacs. The intact cells derive from neuroectoderm and usually are individualized, rarely forming aggregates or clumps of cells. Intact cells have central round to oval nuclei of uniform size and light chromatin, usually with a single nucleolus and lightly basophilic cytoplasm. Neuroendocrine tumors include carotid body tumors (chemodectomas) that form near the angle of the jaw, aortic body tumors (heart base tumors), and pheochromocytomas, which are found in the adrenal glands. Brachycephalic breeds tend to have more of these tumors than breeds.

Tumors of Connective Tissue Origin (Sarcomas) (See Chapter 20)

The cells of spindle cell tumors are similar in appearance to normal connective tissue cells, but they exhibit variable features of malignancy. The cytoplasm is light blue to gray or lightly eosinophilic; it follows the contour of the nucleus and is elongated and oval or streams at both ends to form tail-like points (Figure 7-21). Sometimes the cells are plump and have an angular, pointed cellular end. The nuclear chromatin arrangement and number of nucleoli vary considerably. More cellular tumors tend to have multiple nuclei, multiple nucleoli, and varying shapes and sizes of nuclei and nucleoli. Multinucleation is a characteristic of more aggressive tumors. More malignant, less well-differentiated cells usually are less spindled in appearance. Cells usually appear individually rather than in clusters but may be very close together, making them difficult to distinguish from epithelial tumors. The cells have indistinct cytoplasmic borders, pale blue cytoplasm that may contain a few vacuoles, and large, oval nuclei with prominent, often multiple nucleoli.

Tumors of mesenchymal origin may be difficult to diagnose definitively as to tissue origin. Osteosarcomas usually have a distinctive appearance in that the cells resemble giant plasma cells, with an eccentric nucleus appearing to protrude from one end of the cell (Figure 7-22). The cytoplasm usually contains small eosinophilic granules. Background pink matrix typically is present. Chondrosarcomas and myxosarcomas usually have abundant background matrix (glycosaminoglycans). Liposarcomas have a distinctive appearance in that the cytoplasm contains multiple small, fat vacuoles. Cells from lipomas appear identical to normal fat cells, as described previously. Other spindle cell tumors include fibrosarcomas, hemangiosarcomas, hemangiopericytomas, neurofibromas (peripheral nerve sheath tumors), leiomyosarcomas, rhabdomyosarcomas, and vaccine-induced sarcomas. Selected connective tissue tumors are described in more detail below.

Fibrosarcomas

Fibrosarcomas produce cellular preparations consisting of numerous spindle cells with moderate to marked cellular and nuclear variability, including multinucleation. Eosinophilic to purple granules may be seen in a few cells. A small amount of extracellular, amorphous
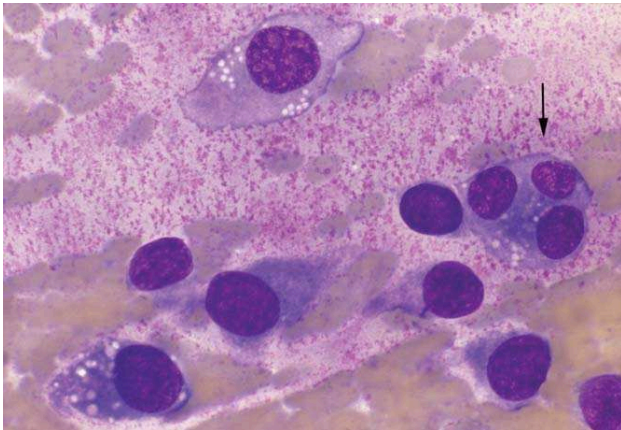
Figure 7-22

Aspirate from an osteosarcoma. Note the cells tending to spindle, the osteoid matrix in the background, and the multinucleated cell *(arrow)*.
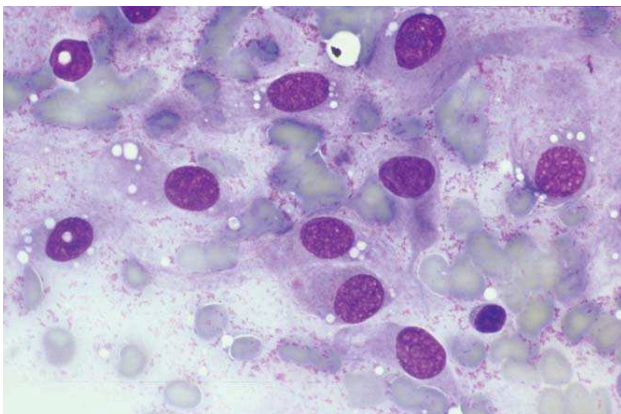
Figure 7-23

Aspirate from a canine fibrosarcoma. Note the cells tending to spindle and the pink glycosaminoglycans in the background.

pink to eosinophilic material that resembles osteoid and is interpreted to be collagen usually is present (Figure 7-23).

Malignant Fibrous Histiocytomas

Cells from a malignant fibrous histiocytoma (MFH) have a more distinctive appearance cytologically in that they consist of multinucleated giant cells in addition to neoplastic-appearing spindled cells. When the cells from an MFH lesion appear more round than spindled, the aspirates can resemble those seen in malignant histiocytosis.

Hemangiosarcomas (See Chapter 33A)

Aspirates of hemangiosarcomas often yield only peripheral blood. Cells are spindle shaped but often quite plump and have multiple features of malignancy. The nuclei
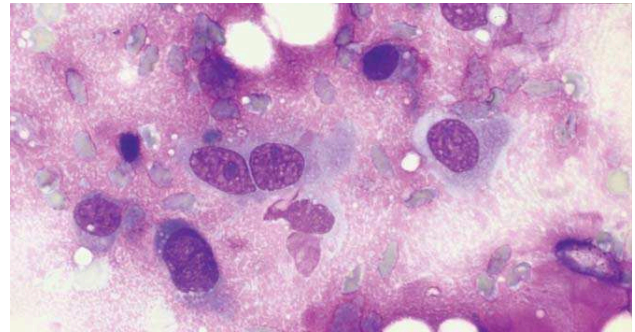
Figure 7-24

Aspirate from a myxosarcoma. A large amount of glycosaminoglycans is present in the background.

usually contain prominent, sometimes multiple nucleoli, and a few cells may be multinucleated. The cytoplasm is light to moderately basophilic and sometimes vacuolated.

Osteosarcomas (See Chapter 23)

Osteoblasts are similar in appearance to plasma cells but are larger, with the nucleus appearing to protrude from one end of the cell. The cytoplasm usually contains small, eosinophilic to magenta granules. Multinucleated cells commonly are present. Background pink matrix (osteoid) typically is present (see Figure 7-22).

Chondrosarcomas and Myxosarcomas

Aspirates of chondrosarcomas and myxosarcomas contain abundant background matrix (glycosaminoglycans) that is light blue to eosinophilic and sometimes stippled, similar to background glycosaminoglycans in joint fluid (Figure 7-24). The cellularity usually is low, and the cells may not spread into a monolayer because of the glycosaminoglycan that adheres to them. This material may cause the cells to align in rows, as they do in other viscous fluids.

Synovial Cell Sarcomas

Synovial cell sarcomas appear similar to other mesenchymal tumors; however, their cellularity usually is quite high, and features of malignancy can be variable. These tumors usually are distinguished by their location and the presence of glycosaminoglycans in the background. Well-differentiated synovial cell sarcomas can be difficult to distinguish from synovial hyperplasia lesions.

Liposarcomas

Liposarcoma cells have a distinctive appearance in that the cytoplasm contains multiple small, fat vacuoles and the nuclei are much larger than those of adipocytes. The cells exhibit features of malignancy and sometimes can resemble cells from a sebaceous cell carcinoma. Liposarcomas are far more cellular and anaplastic then the more common lipomas.
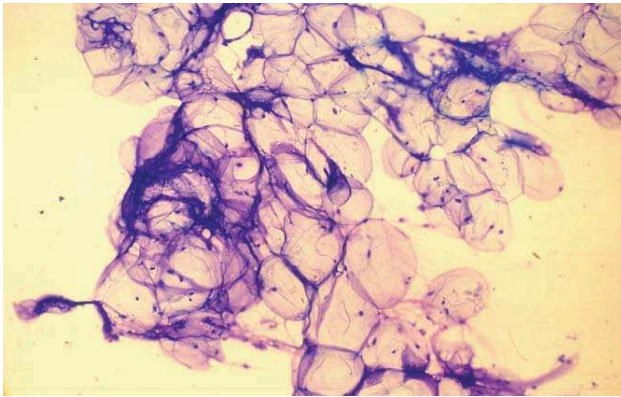
Figure 7-25

Aspirate from a lipoma (low magnification). Note the very small nuclei in the adipocytes.
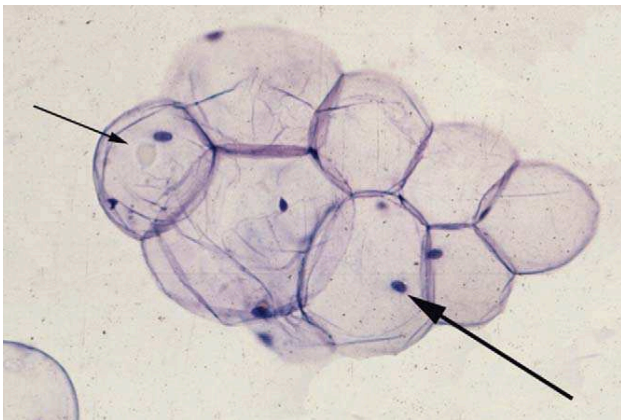
Figure 7-26

High magnification of adipocytes from a lipoma. Note the erythrocyte *(small arrow)* and the nucleus of the cell *(large arrow)*.

Lipomas

Lipomas consist of adipocytes that appear as very large, clear, round cells with very small, dense nuclei (Figures 7-25 and 7-26). They often appear in clusters and may wash off in the alcohol fixative. Numerous free fat droplets may be present. Aspirates of subcutaneous fat and perinodal fat can appear identical to aspirates of lipomas.

Vaccine-Induced Sarcomas

Vaccine-induced sarcomas, which develop at typical vaccine sites in cats, initially are inflammatory and appear similar to other pyogranulomatous inflammatory lesions. However, the distinctive appearance of vaccine adjuvant, which is seen in macrophages and between cells, is highly suggestive of a vaccine-induced lesion. This material is blue to magenta and quite amorphous, similar to ultrasound gel. As these lesions transform into sarcomas,
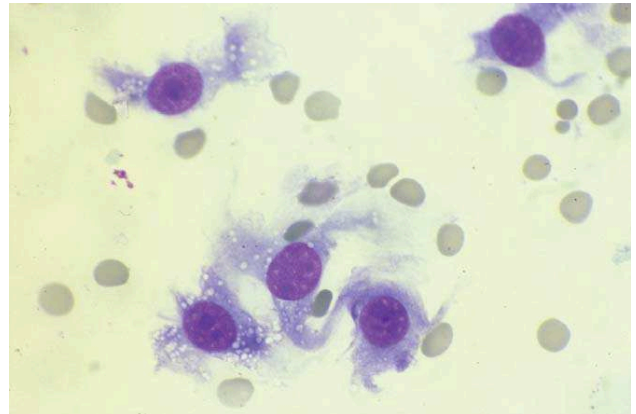
Figure 7-27

Aspirate from a soft tissue sarcoma, likely an hemangiopericytoma or nerve sheath tumor. The cells have a stellate appearance and cytoplasmic vacuolation.

more mesenchymal cells appear; eventually, these cells exhibit many features of malignancy, including very large cell, nuclear, and nucleolar size. Numerous multinucleated giant cells may be present.

Hemangiopericytomas and Nerve Sheath Tumors

Hemangiopericytomas and nerve sheath tumors are common in dogs. Aspirates of these tumors typically have a very high cellularity and consist of distinctly spindle-shaped cells with long tails of cytoplasm extending from both ends of the cell. They may appear singly or in clusters or whorls. The nuclei usually are oval to elongated and have prominent nucleoli. The cytoplasm is lightly basophilic and may contain small, discrete, clear vacuoles (Figure 7-27). These tumors, which commonly are found on the limbs, have a tendency to recur after excision.

Rhabdomyosarcomas

Aspirates of rhabdomyosarcomas tend to exhibit spindle cells in rows. Only rarely can cells be seen in which the cross striations are still visible; in contrast, aspiration of normal skeletal muscle yields muscle fibers in which cross striations are very apparent.

Melanomas (See Chapter 18)

Melanomas are classified as mesenchymal tumors, but their appearance is distinctive in that some cells resemble epithelial cells, some are spindled, and yet others appear to be round and discrete. Malignant melanocytes typically have a large, single "owl's eye" nucleolus in the nucleus. If the cytoplasm contains melanin granules (usually fine, gray, dustlike particles), the cells can be readily identified (Figure 7-28). However, many cells do not contain pigment (i.e., they are amelanotic).
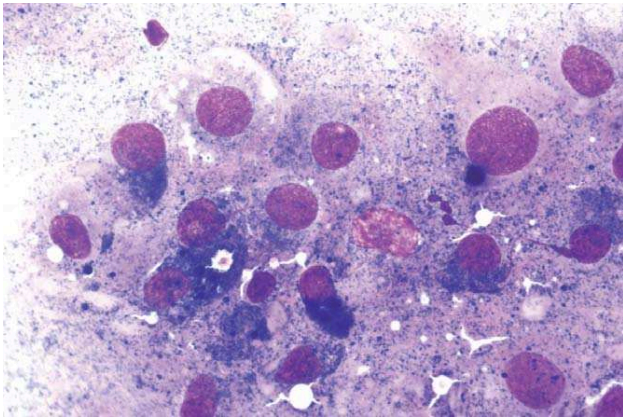
Figure 7-28

Aspirate from a melanoma. Note the variability in nuclear size and the melanin granules in the background and in the cytoplasm of the intact cells.
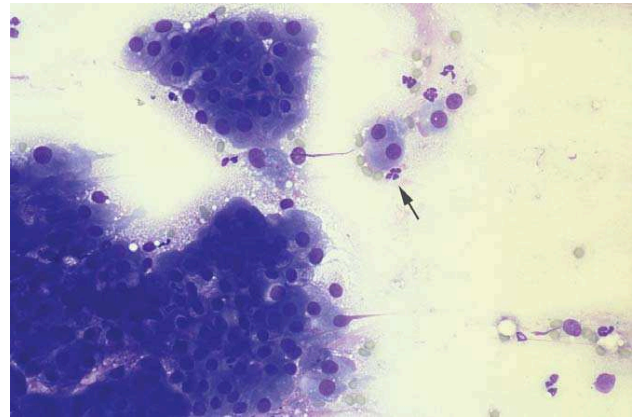
Figure 7-29

Aspirate from a lung carcinoma (low magnification). The cells appear in clusters and sheets, indicating that they are epithelial in origin. The arrow indicates a neutrophil.

Neoplasia of Body Cavity Effusions, the Lower Respiratory Tract, and Intra-abdominal Organs

Thoracic and Abdominal Cavity

Neoplastic effusions may be modified transudates or inflammatory exudates. They are characterized by the presence of neoplastic carcinoma or lymphoma cells, because sarcomas do not readily exfoliate cells. The total protein is variable, generally ranging from 3 to 4.5 g/dl. The cell concentration also is variable, usually ranging from 3000/μl to 30,000/μl. Fluid that has accumulated secondary to a carcinoma may also contain variable numbers of inflammatory cells. Some degree of expertise is required to differentiate carcinoma cells from mesothelial cells. Mesothelial cells line the body cavities, and when fluid accumulates, these cells tend to proliferate and exfoliate into the fluid, where they may continue to replicate. They may appear singly or in clusters of two, four, eight, or 16 cells. Mesothelial cells are large (12 to 30 μm), have a light to dark basophilic cytoplasm, and have single or multiple round to oval nuclei with one or more nucleoli. Cells in mitosis may be seen. The cytoplasmic border may appear to have a pink fringe around it. Lymphoma usually is less difficult to diagnose cytologically than is carcinoma, because lymphomas typically shed large numbers of lymphoblasts, which are not easily confused with other types of cells, such as mesothelial cells.

Cytopathology of the Lower Respiratory Tract

Neoplasia of the lower respiratory tract occasionally can be diagnosed by tracheal washings or bronchoalveolar lavage; however, direct aspiration of lung lesions previously identified by imaging typically is much more

rewarding (Figure 7-29). The cytologic appearance of the various types of neoplastic cells is as described previously. Lung tumors that may be diagnosed by cytopathology include lymphoma, malignant histiocytosis, pulmonary adenocarcinoma, squamous cell carcinoma, metastatic mammary carcinoma, osteosarcoma, and other types of metastatic sarcoma. Of these, carcinomas are the most common. If cilia are seen on the surface of epithelial cells, the cells are unlikely to be malignant.

Malignant histiocytosis sometimes is confused with anaplastic carcinoma, because both these tumor types may have multinucleated giant cells and abundant cytoplasm. However, the cells of anaplastic carcinomas typically adhere to each other.

Hyperplasia of respiratory epithelium is uncommon in dogs, therefore the diagnosis of neoplasia usually is apparent. However, hyperplasia of type II pneumocytes is a common response to interstitial pneumonia in cats, which makes the distinction between hyperplasia and neoplasia more difficult.

Cytopathology of Intra-abdominal Neoplasms

Cytologic material aspirated from enlarged abdominal organs and abnormal masses in the abdominal cavity is commonly used as a diagnostic aid. Although aspiration often can be accomplished by palpation, ultrasonography and magnetic resonance imaging are extremely helpful for localizing specific organs and masses. Samples obtained by ultrasonography may contain ultrasound gel, which has an amorphous appearance microscopically and stains a dark magenta (Figure 7-30). This material may be so abundant on the slide that it interferes with evaluation. For this reason, alcohol, rather than ultrasound gel, should be used when samples are obtained
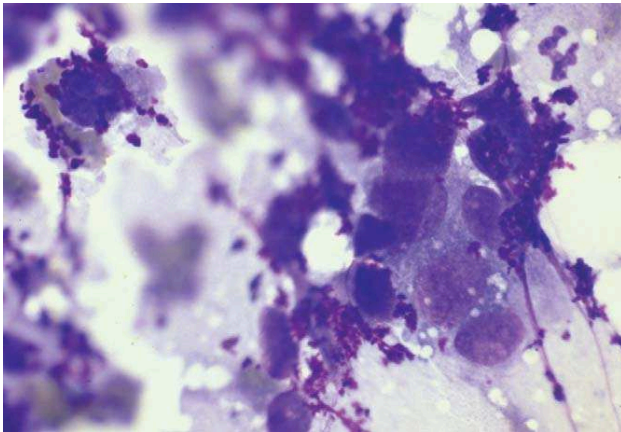
Figure 7-30

Ultrasound gel obscures the hepatocytes in a liver aspirate.

Figure 7-31

Normal-appearing hepatocytes from a canine liver aspirate.

for cytology. If alcohol cannot be used, it is important to keep the biopsy site and fingers free of gel.

The general principles of cytology apply to the interpretation when material from any abdominal mass or organ is examined.[9] The attempt usually is made to determine whether the tissue is normal and, if not, whether inflammation or neoplasia is present. An additional complication in the interpretation of aspirates from the abdominal cavity is that normal structures, usually of epithelial origin, may be aspirated inadvertently. These normal epithelial cells must then be distinguished from neoplastic cells. The cytologic appearance of cells and the classification of neoplasms are the same as discussed previously. Some specific types of intraabdominal neoplasms are discussed briefly in the following sections.

Liver. Hepatomegaly and the presence of areas of differing echogenicity are the primary reasons for liver aspiration.[10-12] Suspicion of neoplasia or inflammation in the liver also is an indication. Nodular lesions can be aspirated with the aid of imaging procedures such as ultrasonography. The only relative contraindications are abnormal hemostasis, caused either by thrombocytopenia (platelet count under 30,000/μl) or by decreased coagulation factor activity, and suspicion of a hemangiosarcoma, which may rupture if lacerated during aspiration. Good technique is critical for obtaining diagnostic liver aspirates.

Excellent samples can be obtained using the method of Menard and Papageorges.[12] Briefly, a 22 gauge, 1.5- to 3.5-inch needle is connected to a 12 ml syringe by an 84 cm, flexible extension set for intravenous lines. The syringe is prefilled with air. The needle hub is held like a pen to allow precise manipulations, and the needle is positioned in the liver. The tip is moved rapidly back and forth eight to 10 times within the same path.
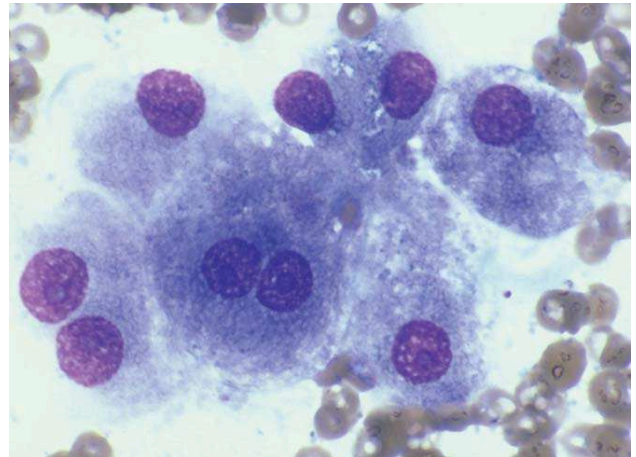
The needle then is withdrawn, and the biopsy material is expelled immediately onto a glass slide using the air-filled syringe. A gentle squash preparation technique is used to make the films. This technique keeps the liver cells at the center and displaces blood to the periphery.

Normal hepatocytes are uniform, large, round to oval cells with abundant basophilic, somewhat granular cytoplasm. Cells contain one or two round, centrally located nuclei with a single prominent, pale blue nucleolus (Figure 7-31). Normal hepatocytes often have a small amount of dark blue-black pigment. This pigment may be a type of bile pigment or occasionally may be lipofuscin, commonly seen in old cats, which is more blue in color than bile pigment. Hemosiderin may also be seen in hepatocytes and usually is golden brown.

Hepatocytes may occur singly or in clusters. Very infrequently, rectangular, crystalline, clear inclusions may be seen in the nuclei of some hepatocytes; these have no known significance.[13] Biliary epithelial cells may also be seen in normal aspirates; these are small cells that are uniform in size and have round nuclei and a relatively small amount of pale blue cytoplasm. Other cells occasionally observed in small numbers in aspirates from normal livers include mast cells, macrophages (Kupffer's cells), lymphocytes, and neutrophils. Mesothelial cells from the surface of the liver frequently are seen in liver aspirates and should not be confused with neoplastic epithelial cells, fibroblasts, or biliary epithelium.

Nonneoplastic abnormalities that can be diagnosed by cytology include cholestasis, hepatic lipidosis, increased glycogen storage, hepatocellular degeneration, various types of inflammation, extramedullary hematopoiesis, copper-associated hepatopathy, and lysosomal storage disorders.[14]
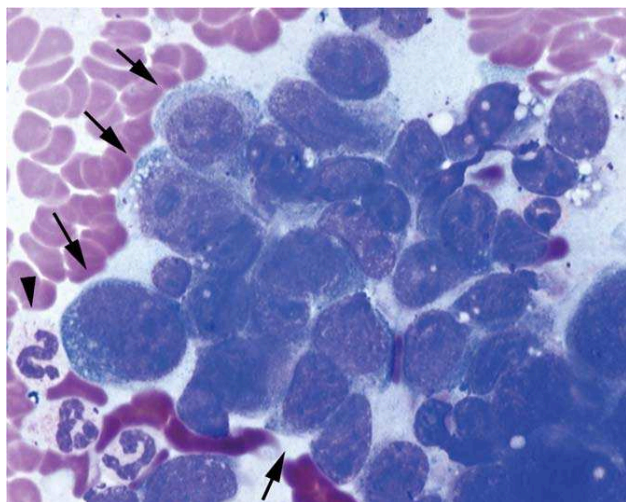
Figure 7-32

Aspirate from an hepatocellular carcinoma that was also infected and inflamed. Neoplastic hepatocytes are indicated by arrows. Note the cellular crowding, large multiple nucleoli, and decreased amount of cytoplasm compared to that of normal hepatocytes. The arrowhead indicates a neutrophil.

Primary Neoplasia of the Liver. Primary neoplasms of the liver that may be diagnosed by cytology include hepatocellular adenomas, hepatocellular carcinomas, bile duct carcinomas, and neuroendocrine tumors. Neoplastic hepatocytes (hepatocellular carcinoma) may resemble normal hepatocytes to some extent, depending on their degree of differentiation. However, unless they are very well differentiated, they exhibit features of malignancy, such as cellular crowding, multiple nucleoli, variation in nuclear size, a higher nucleus-to-cytoplasm ratio, and variation in cell size. Malignant hepatocytes usually are distinguishable from hyperplastic hepatocytes. Occasionally hepatocellular carcinomas are so poorly differentiated they cannot be distinguished from metastatic carcinomas (Figure 7-32). In some aspirates from hepatic tumors, numerous capillaries can be seen among the neoplastic cells.

Malignant biliary epithelial cells (bile duct carcinoma cells) are similar to any other malignant epithelial cell, with cytologic features of malignancy. Biliary cells are smaller than hepatocytes and have less cytoplasm.

Neuroendocrine tumors or carcinoids are derived from the amine precursor uptake and decarboxylation (APUD) cells of the biliary system. The cells are similar to those of other neuroendocrine tumors. Because these cells normally are quite fragile, numerous naked nuclei usually are seen. Intact cells are small and have round, central nuclei. The chromatin usually is condensed, the nucleoli are indistinct, and the cytoplasm is moderate to abundant and usually pale. Cytoplasmic vacuoles may be present.

Metastatic Neoplasia of the Liver. The cells of metastatic carcinomas have malignant features as described earlier, including large size, cell-to-cell relationships (cohesiveness), variability in cell size, variability in nuclear size, prominent nucleoli, basophilic cytoplasm, and perinuclear cytoplasmic vacuoles, especially in secretory cells. Commonly the tissue of origin cannot be determined. Metastatic endocrine and neuroendocrine tumors appear as described previously.

Sarcomas may originate in the liver, but they more commonly are metastatic. Sarcoma cells are spindle shaped and have oval nuclei and prominent nucleoli. The cytoplasm is basophilic and may be vacuolated. The cells are not cohesive, and they may be uniform or variable in size and shape. Sarcoma cells do not exfoliate as readily as carcinoma cells, but they are readily observed on imprints of surgical biopsies. These cells should not be confused with fibroblasts, which may be present in animals with hepatic fibrosis. Hemangiosarcomas are the most common sarcomas and usually have metastasized from the spleen. Evidence of previous hemorrhage (erythrophagocytosis and hemosiderin-laden macrophages) and polychromatophilic erythrocytes may be observed, as may abnormally shaped erythrocytes (acanthocytes), commonly seen in blood films from dogs with hemangiosarcoma.

Discrete cell neoplasms may be found in the liver. Lymphoma, plasma cell tumors, mast cell tumors, and malignant histiocytosis are somewhat common neoplasms of the liver. They usually are relatively easy to diagnose, because neoplastic cells from these types of tumors customarily are diffusely distributed. Liver aspirates containing large numbers of small lymphocytes are more difficult to interpret, because the lymphoid population may represent either an inflammatory process or a small cell lymphoma. Surgical biopsy and histopathology, as well as blood, bone marrow, and lymph node cytology and PCR, can be used to differentiate the two processes.

Myeloproliferative disease may be present in the liver and spleen. If the cells are somewhat differentiated, the presence of recognizable progranulocytes with pink cytoplasmic granules aids diagnosis. If the tumors are undifferentiated, the immature cells may resemble lymphoblasts, and PCR, special cytochemical stains, and bone marrow or blood examination are required to distinguish between the types of cells. Myeloproliferative disease sometimes is misdiagnosed as extramedullary hematopoiesis or inflammation.

Pancreas. The pancreas typically is aspirated only if pancreatic masses are detected. As with other organs, inflammation, neoplasia, and cystic structures usually can be differentiated based on cytologic findings. Neoplasms of the exocrine pancreas (pancreatic adenocarcinoma) have characteristics similar to those of other adenocarcinomas, including variability in cell size and nuclear

size, prominent nucleoli, a high nucleus-to-cytoplasm ratio, and a basophilic, vacuolated cytoplasm. Malignant ascites may often accompany exocrine pancreatic adenocarcinomas.

Neoplasms of the endocrine pancreas usually are tumors of the beta islet cells and are referred to as *insulinomas*, *beta cell tumors*, or *islet cell tumors*. They develop most often in large breed, older dogs and in ferrets. They are rare in cats. Their appearance is similar to that of other endocrine tumors, such as thyroid gland tumors. Aspirates typically consist of numerous naked nuclei in a background of blue cytoplasm. However, unlike thyroid tumors, the cytoplasm of intact cells commonly is filled with small, discrete, clear vacuoles.[1] The nuclei usually contain a single, prominent nucleolus. The cells typically are quite uniform, although some variability in cell and nuclear size may be present. Most of these tumors in dogs are malignant in behavior and metastasize to regional lymph nodes and the liver. Most are functional and secrete excess insulin, resulting in hypoglycemia that may be quite severe (less than 50 mg/dl).

Adrenal Gland. Adrenal gland tumors may arise from the adrenal cortex or the medulla. Tumors of the cortex produce excess glucocorticosteroids, resulting in hyperadrenocorticism. Tumors of the medulla produce excess catecholamines. Adrenocortical enlargement usually occurs secondary to pituitary tumors in dogs, in which case it is bilateral. However, adrenocortical tumors occur in fewer than 50% of dogs with hyperadreno-corticism, with approximately equal numbers of adeno-mas and adenocarcinomas. Aspiration of these masses usually is accomplished using ultrasonography.

The cells of adrenocortical adenomas resemble normal or hyperplastic secretory cells of the adrenal gland. They also are similar to cells from aspirates of other endocrine organs, with most cells appearing as naked nuclei. The cytoplasm usually is moderately basophilic and often contains numerous small, discrete vacuoles. The nuclei are round and uniform and typically have a single, prominent nucleolus. Cells from adenocarcinomas may be more variable in size and have more prominent, multiple nucleoli. However, these cells may also resemble adenoma cells.

Tumors of the adrenal medulla (pheochromo-cytomas or chromaffin cell tumors) are rare and may be aspirated with the aid of ultrasonography. Cells from these tumors are similar to those from other neuroen-docrine tumors, consisting of clusters of fairly uniform cells with numerous naked nuclei. The cytoplasm is light blue and may contain pale basophilic granules. The nuclei are round and have a single, small nucleolus. Significant features of malignancy are not typically present. Significant hypertension secondary to pheochromocy-toma is a relative contraindication to aspiration.
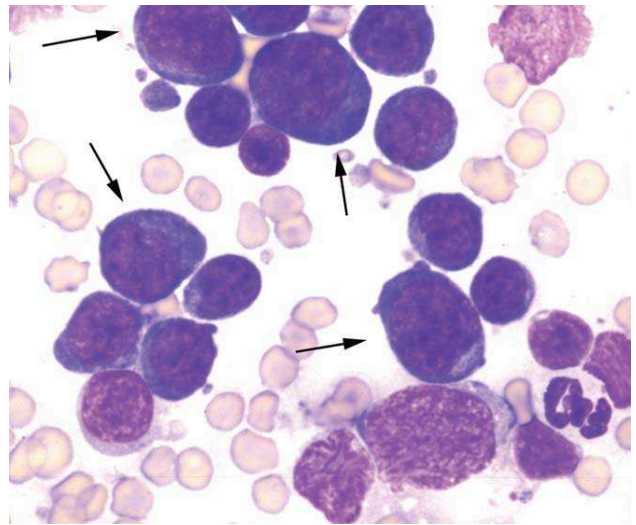
Figure 7-33

Aspirate from a mass in the intestine of a cat. The numerous lymphoblasts *(arrows)* are diagnostic for lymphoma.

Gastrointestinal Tract. Although samples for cyto-logy of the gastrointestinal (GI) tract often are obtained using endoscopy, large masses involving the GI tract may be palpated and aspirated through the abdominal wall, with or without the aid of ultrasound imaging. Intestinal epithelial cells normally are quite uniform and have round nuclei and a basophilic cytoplasm that may appear confluent. Mucus-producing goblet cells may also be seen. Goblet cells have large, clear vacuoles in their cytoplasm and may contain magenta mucin granules. Normal-appearing lymphocytes may be aspirated from lymphoid follicles or may be present in animals with lymphocytic enteritis. A few large, granular lymphocytes may also be observed. Inflammatory lesions may be present, with variable numbers of neutrophils, macrophages, mast cells, lymphocytes, and eosinophils.

Neoplasia of the GI tract often can be diagnosed using transabdominal aspiration. Lymphoma is charac-terized by large numbers of lymphoblasts (Figure 7-33). Adenocarcinoma of the GI tract is similar in appearance to other types of adenocarcinoma (Figure 7-34).

Adenocarcinoma of the GI tract may elicit a scirrhous reaction in which numerous fibroblasts are also present. Sarcomas, such as leiomyosarcoma, occasionally are diagnosed by cytology.

Kidney. The kidneys often are aspirated because they are large or abnormally shaped.[15,16] Neoplastic kidneys, especially those with lymphoma, are likely to provide diagnostic material. Renal tubular epithelial cells usually are present in the sample. These cells are approximately 20 μm in diameter and have abundant,
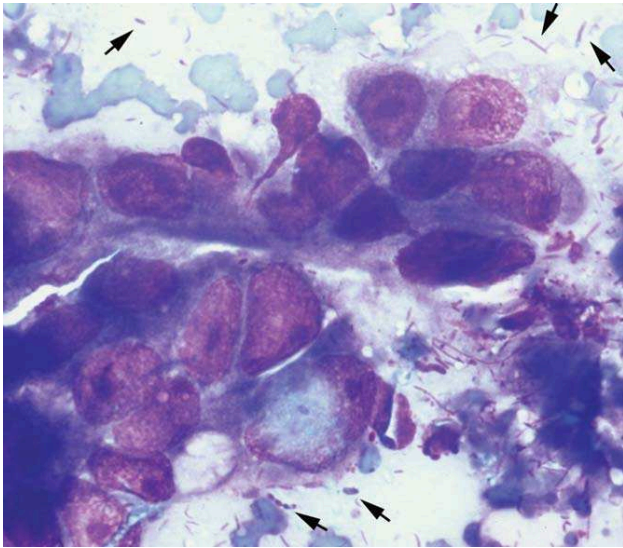
Figure 7-34

Aspirate from a mass in the intestine of a cat. Epithelial cells with large nucleoli are suggestive of an intestinal adenocarcinoma. Numerous bacteria of various types are present in the background *(arrows)*.
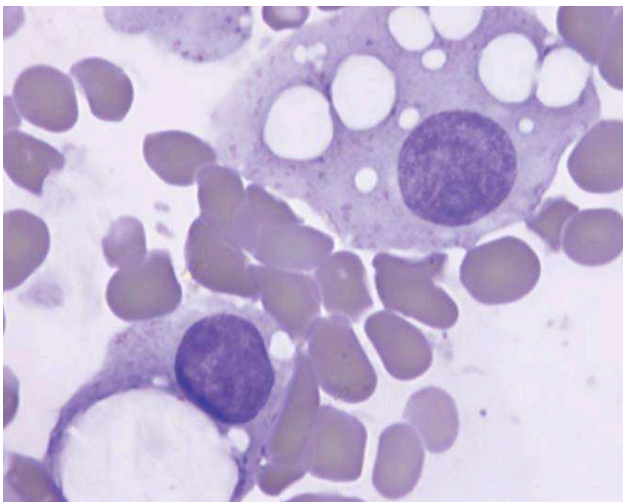
Figure 7-35

Very high magnification of normal renal epithelial cells from a feline kidney aspirate. Feline renal epithelial cells commonly contain large vacuoles that presumably are lipid.

light blue cytoplasm and round nuclei with a single, distinct nucleolus (Figure 7-35). The cytoplasm may contain dark granules, and in cats, it often is vacuolated because of the presence of lipid. Blood contamination is common, and platelet clusters may be seen.
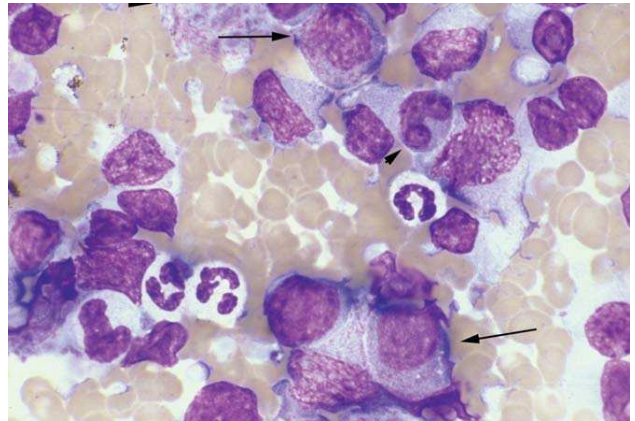
Figure 7-36

Splenic aspirate from a dog with granulocytic leukemia. Progranulocytes are indicated by arrows. The arrowhead indicates a metamyelocyte. A differential diagnosis would be extramedullary hematopoiesis with a preponderance of early myeloid precursors.

Spleen. Cytologic examination of aspirates from the spleen typically is performed for patients with splenomegaly. Cytopathology of the spleen has been described in detail elsewhere.[17-21] Common causes of splenomegaly include hyperplasia, inflammation, extramedullary hematopoiesis, and neoplasia. Common types of neoplasia include lymphoma, myeloproliferative disorders (Figure 7-36), mast cell tumors, malignant histiocytosis, and hemangiosarcoma. Fibrosarcoma, leiomyosarcoma, and metastatic neoplasia may also be seen. Splenic enlargement may be diffuse and symmetric or localized and asymmetric. A contraindication to splenic aspiration is hemangiosarcoma, which usually is asymmetric; the tumor may rupture or implant the peritoneum when aspirated. Normal cytologic preparations from the spleen consist of blood, normal splenic stromal cells (fibrocytes), and small lymphocytes.

Prostate. Normal prostatic cells are cuboidal to columnar and uniform, varying from approximately 10 to 15 μm in diameter. The nuclei are round to oval and are basilar in columnar cells. The nucleoli are small and inconspicuous, and the cytoplasm is finely granular and basophilic. These cells are easily differentiated from transitional epithelial cells, which are larger and have lighter-staining cytoplasm. Cells from hyperplastic prostates almost always appear very similar to those from normal prostates. Numerous neutrophils and variable numbers of macrophages are present in material from inflamed prostates (Figure 7-37). Lymphocytes and plasma cells are seen infrequently. Many of the neutrophils exhibit karyolysis and cytoplasmic vacuolation in the presence of bacteria.
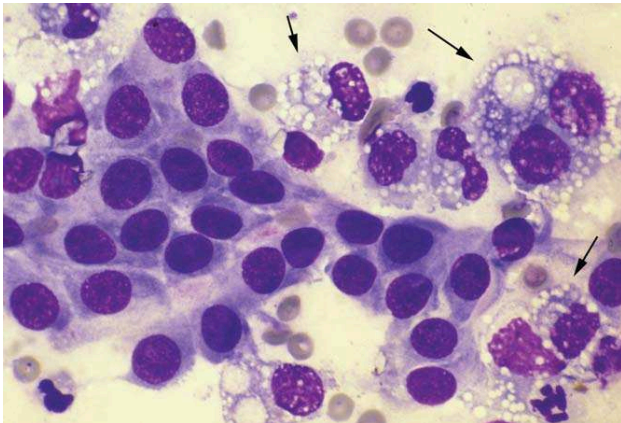
Figure 7-37

Aspirate from an inflamed prostate gland. Macrophages are indicated by arrows. The prostatic epithelial cells present in the large sheet are normal in appearance.

Figure 7-38

Aspirate from a prostatic carcinoma. Note the large, multinucleated cells and the variability in cell size. Numerous neutrophils are present, indicating concurrent suppurative inflammation.

Large, pleomorphic cells (20 to 80 μm) usually are present in aspirates from prostatic tumors. The nuclei are large and round to oval and contain large, prominent, usually multiple nucleoli. The cytoplasm frequently is vacuolated and often contains eosinophilic areas that represent secretory material. Mitotic figures and multinucleate cells frequently are observed (Figure 7-38). Other features of malignancy also are often evident, such as nuclear molding, abnormal mitoses, abnormal chromatin structure, giant cell formation, free nuclear fragments, an increased nucleus-to-cytoplasm ratio, and incomplete separation of cells after division. Transitional cell carcinoma cells are cytologically similar to prostatic adenocarcinoma cells, and the two types of carcinomas often are not differentiated by cytologic examination.[22,23] Prostatic tumors may be missed during aspiration unless the gland is diffusely involved or imaging is used.

Cytopathology of Bone Marrow Neoplasia

A detailed discussion of bone marrow neoplasia is beyond the scope of this chapter; it is discussed in detail elsewhere.[24] Briefly, leukemia, a neoplastic proliferation of hematopoietic cells in the bone marrow, is defined by the presence of neoplastic blood cells in the peripheral blood or bone marrow and is classified broadly into myeloproliferative and lymphoproliferative disorders (see Chapter 31). Diagnosis of these disorders is based on the presence of characteristic blast cells in the blood or bone marrow (or both) and associated hematologic abnormalities. Specific cell types are identified by their morphologic appearance in Wright's-stained blood and bone marrow films, cytochemical staining properties, electron microscope appearance, and monoclonal antibody binding to surface antigens.

In some cases, cells may appear so morphologically undifferentiated that classifying the disorder into either the myeloproliferative or lymphoproliferative category may be difficult. Myeloproliferative leukemias include neoplastic proliferation of erythrocytes, granulocytes, monocytes, and megakaryocytes. Multiple cell lines may be neoplastic if the affected stem cell is multipotential; an example is myelomonocytic leukemia, in which both neutrophils and monocytes have been neoplastically transformed. Lymphoproliferative disorders of bone marrow include acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma.
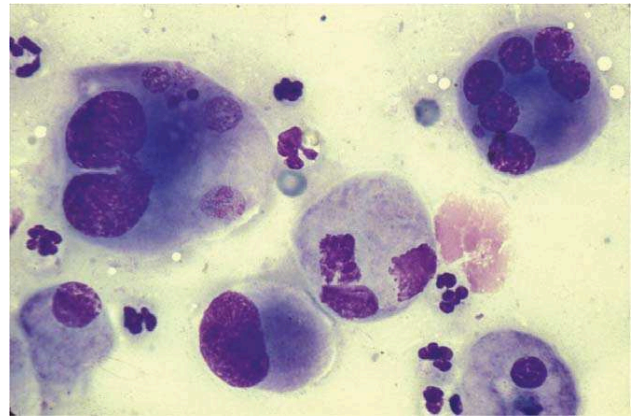
Leukemias are further classified as acute or chronic based primarily on the maturity or degree of differentiation of the neoplastic cells and on the clinical course. The neoplastic cells in acute leukemias are immature (blasts), and the patient survival time usually is quite short. By definition, the presence of 30% or more blast cells in the marrow is diagnostic of acute myeloid leukemia. The percentage of blast cells in the blood, on the other hand, is quite variable in these patients. Chronic leukemias are characterized by the predominance of mature, more well-differentiated cells in the blood and marrow, and the patient survival time usually is longer. Neoplastic cells commonly can be found in organs other than the marrow in patients with leukemia. The spleen frequently is involved, and the liver and lymph nodes also may contain neoplastic cells. Other neoplastic disorders involving bone marrow include mast cell neoplasia, malignant histiocytosis and, in rare cases, metastatic carcinoma and sarcoma.

Metastatic Neoplasia of the Bone Marrow. In rare cases, epithelial and mesenchymal tumors may metastasize to the bone marrow. Epithelial tumors (carcinomas) tend to form groups of cohesive cells that are easy to distinguish from normal hematopoietic cells. Metastatic sarcomas, which are more difficult to diagnose, are characterized by the presence of large, discrete, spindle-shaped

cells that exhibit multiple features of malignancy. These cells must be distinguished from fibroblasts that may be observed in myelofibrosis.

WHERE SHOULD CYTOPATHOLOGY BE PERFORMED?

Veterinarians may decide either to have most cytologic examinations performed and evaluated in house or to send samples to a diagnostic laboratory. However, with a few disorders the results are needed immediately, and the cytologic examination should be performed in the clinic setting. These disorders usually are serious bacterial infections, such as may be seen with pyothorax or bacterial peritonitis.

The diagnosis of neoplasia is rarely a medical or surgical emergency, although patient care and the prognosis can be expedited by prompt cytologic diagnosis. Mast cell tumors and soft tissue sarcomas are examples of neoplasms that are not necessarily emergencies, but for which a knowledge of the tumor type before surgery can be advantageous. These tumors should be excised widely to prevent recurrence. Some types of neoplasia are quite difficult to diagnose and should be interpreted by an experienced cytopathologist. These include carcinoma and mesothelioma in abdominal effusions, owing to the difficulty of distinguishing reactive mesothelial cells from neoplastic mesothelial and epithelial cells. Other examples of cytologic determinations that are inherently difficult, usually because malignant and benign processes are difficult to distinguish in these tissues, include nasal cytology, mammary gland cytology, urinary bladder cytology, liver cytology, and cytology of most other intra-abdominal organs. Small cell variants of lymphoma can be very difficult to distinguish from lymphoid hyperplasia.

The risks of aspiration cytology are low but not zero.[25]

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Molecular Diagnostics

Chand Khanna and Melissa C. Paoloni

OVERVIEW OF MOLECULAR TECHNIQUES USED IN ONCOLOGY

Since the mid-1980s, advances in the fields of molecular biology and genetics have changed our understanding of the biology of cancer. Technological advances now provide the opportunity for applying this advanced understanding to the clinical arena in the form of novel diagnostic tests and management strategies. Molecular approaches for cancer diagnosis are now part of the standard of care for most human patients.[1,3,4] Technological advances have reduced the unit cost for many of these approaches, and their use and commercialization is increasingly taking hold within the veterinary field. It is now feasible to assess proteins, RNA, DNA, or their metabolites in tumors, blood, saliva, and urine to more accurately classify and detect various forms of neoplasia.[2a,2b] The goal of this chapter is to review several molecular techniques useful in the diagnosis and classification of cancer.[3] It is likely that advanced molecular methodologies and diagnostics will continue to improve and become increasingly inexpensive and simpler to use.

METHODS FOR DNA EXAMINATION

DNA (deoxyribonucleic acid) represents the genetic code of all species. This code consists of a series of continuous nucleic acid sugar strands linked through hydrogen bonds. This series of nucleic acids takes on a tertiary folded structure through modification by binding proteins, called histones.[4] The folded and wrapped DNA strand is packaged within the chromosomes of the cell. The earliest techniques used to assess the genetic changes of cancer were those that defined gains, losses, or structural changes in chromosomes, referred to as cytogenetics.[5,6]

Cytogenetics

Historically, these techniques involved the examination of metaphase preparations made from chromosomes. Metaphase preparations were then stained (banded) to help in the identification of distinct chromosome morphologies. Using these techniques, detection of gross abnormalities in chromosome number (ploidy) and the presence of chromosomal translocations were possible and led to the identification of genes associated with tumor development and progression. Cytogenetic analysis has been most useful in the clinical assessment of leukemias, where metaphase preparations are relatively easy to develop from whole blood samples.[6] For most human leukemias, cytogenetic descriptors are used to define distinct subgroups into prognostic groups and to guide treatment decisions. The use of cytogenetic approaches in the management of companion animals has been limited due to the difficulty in using conventional chromosomal banding to identify canine chromosomes. The development of chromosome-specific "paints" that allow the identification of specific canine chromosomes has improved the opportunity to apply cytogenetic descriptors to canine cancers.[7–12] Using these techniques, Breen and colleagues have identified a chromosomal translocation in many canine and feline cancers that includes a chromosomal translation syntenic for the bcr-abl Philadelphia chromosome in humans (personal communication, M. Breen). For the most part, traditional cytogenetic techniques, including the use of chromosome-specific paints, are labor intensive and have been replaced by alternative modalities. Comparative genomic hybridization (CGH) and, more recently, CGH arrays can define gains and losses in chromosome number within tumor specimens rapidly and with highly reproducible results.[8] A broader discussion of array-based diagnostics is provided in the section on RNA-based detection approaches, presented later in this chapter. CGH arrays

are now routinely used to assess gains and loss for most human cancers. A first-generation CGH array has been developed for use in dogs.[11] Using this canine CGH array, a study has been undertaken into the chromosomal changes within a group of dogs with lymphoma (personal communication, K. Lindblad-Toh). Such genetic characterization of a diverse group of cancers, with distinct biological behaviors but similar histologic descriptions, will significantly improve opportunities to target specific therapies and management strategies to distinct biological subgroups of these diseases.

As specific abnormalities are defined in canine cancer, it is likely—as has been the case in human cancers—that simplified and often PCR-based (polymerase chain reaction; Figure 8-1) technologies will be adapted to rapidly identify these abnormalities as prospective diagnostic tests.[13–15] The use of PCR, using primer sets that bridge the translocation breakpoints, has largely replaced cytogenetic assessment of reciprocal translations seen in most translocation-positive cancers found in humans. Assessment of the size of a PCR product—and the opportunity to confirm the product identity by Southern blot or by sequence analysis—allows identification of not only the presence of a translocation but also the specific translocation. The presence of a translocation can also be verified using fluorescent *in situ* hybridization (FISH), with chromosome-specific paints.[8]

Utility of the Release of the Canine Genome Project

The ability to use the genetic code to understand physiology and pathophysiology has been dramatically changed through the recent release of the full genome map (DNA sequence) of humans in 2000 and the release of a full draft of the canine genome map in 2005.[16] The availability of this full genetic sequence has increased the speed with which most candidate-based evaluations of gene expression can be undertaken (i.e., Southern blot and PCR). It has also provided the data needed to develop high throughput and non-candidate-based methodologies.

Figure 8-1

PCR/RT-PCR method boxes. RT-PCR involves the use of reverse transcriptase (RT) to make a complementary strand cDNA (complementary DNA) from a target mRNA strand. The cDNA is then amplified by traditional PCR methods. The Taq DNA polymerase is able to create a new DNA strand from specific DNA primers, complementary to the target gene of interest, in a reaction that includes the RT template sequence and oligonucleotides. The three steps involved include denaturation (melting) of the double-stranded DNA at high temperatures, incubation at lower temperatures to allow for hybridization of the primers, and then synthesis in which new DNA strands are built. Repetition of these cycles multiple times allows for amplification of even very small amounts of cDNA to detectable levels. Direct sequence information for regions flanking the primers is necessary for successful amplification. Sequences of 200–1000 bp can be amplified without protocol modification. Ethidium bromide staining and gel electrophoresis are required for visualization of the PCR products (seen as bands again based on their size). These products are then sequenced to confirm their identity.

Tumor tissue

Extract tumor RNA

Reverse Transcription: RNA to DNA

PCR: Denaturation, Hybridization, Amplification

Gel Electrophoresis of PCR products

Standard Curve Method

Figure 8-1, cont'd

Quantitative RT-PCR defines the reaction products for each sample in every cycle, allowing for the generation of a standard curve and copy number calculation. PCR products are detected through the generation of a fluorescent signal. The fluorescent signal can come from the growing double-stranded DNA product of the PCR reaction, either measured from the strand itself or with fluorescently labeled PCR probes. Results are quantified based on the standard curve method or the comparative threshold method. The standard curve method involves the generation of a curve of a known quantity of RNA to which mRNA of unknown concentrations are referenced. The comparative threshold approach compares the threshold concentrations of the samples with the concentration of a control such as an endogenous housekeeping gene that is amplified simultaneously with the mRNA of interest. An ideal internal control for quantitative RNA analysis is 18S rRNA, because it is consistently expressed across tissues and treatments.

PCR-Based Techniques

PCR-based diagnostic tests have become available for the assessment of lymphoid malignancies in the dog and cat.[17] PCR primer sets have been designed to amplify the common regions of the T-cell receptor (TcR) and the immunoglobulin receptor (IgR) in dogs and cats. Amplification of PCR products from genomic DNA, purified from whole blood or tissue, yields T- or B-cell-specific products. The size of the resultant product is defined in part by the specific recognition domain for the T- or B-cell clone from which the reaction emerged. The resolution of a single TcR or IgR band from a test sample suggests a clonal population of lymphocytes in a sample (Figure 8-2).[17–19] Conversely, the presence of multiple bands may suggest a polyclonal lymphoid

population consistent with an inflammatory reaction. Such tests of clonality can support the presence of lymphoid neoplasia versus inflammation and can assist in defining the immunophenotype of a lymphoid cancer. However, other confirmatory diagnostic data should be included in the assessment of these data. False positive, single-band products may be seen in some inflammatory responses. The use of PCR to assess lymphoid clonality may be complemented by parallel flow cytometry reactions using antibodies against specific lymphoid markers.[19–24] The clinical application of these PCR and single-cell methods of detecting and diagnosing lymphoid cancers in dogs and cats may also become useful in the assessment of minimal residual disease and defining early recurrence for some presentations of these cancers.

PCR clonality

Germline — Monoclonal

PCR product of:
Germline V-D-J
Polyclonal V-D-J
Monoclonal V-D-J

Sizing gel electrophoresis
EtBr staining

Polyclonal
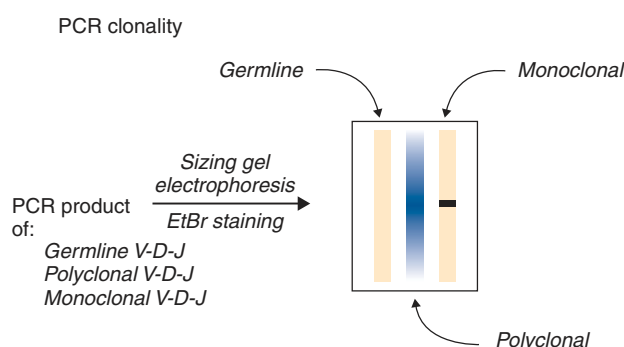
Figure 8-2

PCR clonality. Clonality is the hallmark of malignancy. PCR is able to detect the clonal rearrangement of the antigen receptor immunoglobulin or T-cell receptor genes. The ability of lymphocyte receptor V, D, and J gene segments to rearrange generates the receptor diversity that is the hallmark of the immune system. Molecular positivity for B cell clonality is defined as a single band on gel electrophoresis illustrating monoclonal origin of the B cell receptor. PCR assessment of lymphoid tissue, bone marrow, or peripheral blood is possible and facilitates the diagnosis of lymphoma in these puzzling clinical scenarios. This methodology can also be used to detect minimal residual disease (MRD) in lymphoid malignancies, possibly identifying patients who require further therapy.

SNP Analysis

A great deal of interpatient variability in the biology of cancers is based on the presence or absence of mutations in a cancer. However, small differences in host genes—referred to as single nucleotide polymorphisms (SNPs)—have been shown to be important in defining these interindividual differences in disease progression and response to treatment.[25–27] SNPs can result in a change in the structure or function of a gene. SNPs are considered to be distinct from mutations since they are present in a subset of the population and do not cause disease themselves. A number of SNPs in genes that function in hepatic metabolism in the human population have been identified and can predict increased risk for toxicity to some drugs. Similarly, SNPs in other genes have been shown to predict an increased risk for a more aggressive course of metastatic progression once a patient develops a cancer.[26–30] The release of the canine genome sequence has made possible the high throughput detection of SNPs—using an array platform—within a population of dogs.[31,32] It is likely that SNP-based diagnostics will emerge from these discovery efforts and will contribute to the individualization of therapy of dogs and cats with otherwise similar diseases. SNP-based diagnostics are also helpful in providing an opportunity to attempt to predict or define individual differences seen between patients treated in the same way for the same disease. These differences may include differences in toxicities experienced after receiving a treatment, differences in beneficial response to a therapy, or differences in progression rates for patients with a similar disease stage. These forms of individualized therapy are on the horizon for both human and veterinary cancer patients.

METHODS FOR RNA EXAMINATION

Introduction

The complete genetic code, or DNA sequence, is present within every cell in the body. The effective genetic information that uniquely defines each cell type within the body is defined by the genes expressed (transcribed) as messenger RNA (mRNA). Within the full complement of genomic DNA, sequences known as exons remain, whereas the noncoding or extraneous regions, known as introns, are excluded in the transcription of mRNA. As such, the expression of mRNA is more responsible for the phenotype of a cancer than assessment of genomic DNA. Most molecular diagnostic tests are based on the detection of mRNA. The following section will outline methods of mRNA assessment including Northern blots, RT-PCR, and expression microarrays.

Northern Blot

Northern blotting was developed using procedures similar to those first described for DNA quantification by Edward Southern and is a method used to study gene expression (mRNA). A Northern blot is used to define and quantify the presence of specific mRNA sequences within cells or tissues (Figure 8-3).[33,34] The utility of a Northern blot is greatest when used to detect the expression of an abundant gene within a tumor sample, especially where antibodies for the resultant protein are not available. An example of this utility is the metastasis suppressor gene, Nm23, which was evaluated in lung cancer via Northern blot analysis and found to correlate with differentiation but not with stage of disease.[35]

RNase Protection Assays

Some techniques for nucleotide or protein evaluation are valuable in experimental research designs to characterize cancer; however, they have less defined clinical applications. One such example is the RNase protection assay, heralded as a more sensitive means to detect RNA transcripts within tissues or cells than Northern blotting. Total RNA from a cell or tissue is hybridized to radiolabelled RNA probes complementary to the coding region of a gene of interest. The result of this hybridization is a RNA probe-RNA target duplex. The hybridization

RNA extracted

Electrophoresis separates by size, weight, isoelectric point

weight

Paper towels

GEL

Wet paper towels

Blotted onto Nitrocellulose

Radioactive probe hybridizes to
complementary sequences of RNA

base
8,000
5,000
4,000
3,000
2,000
1,500
1,000
500
200

Bands then detected by autoradiography
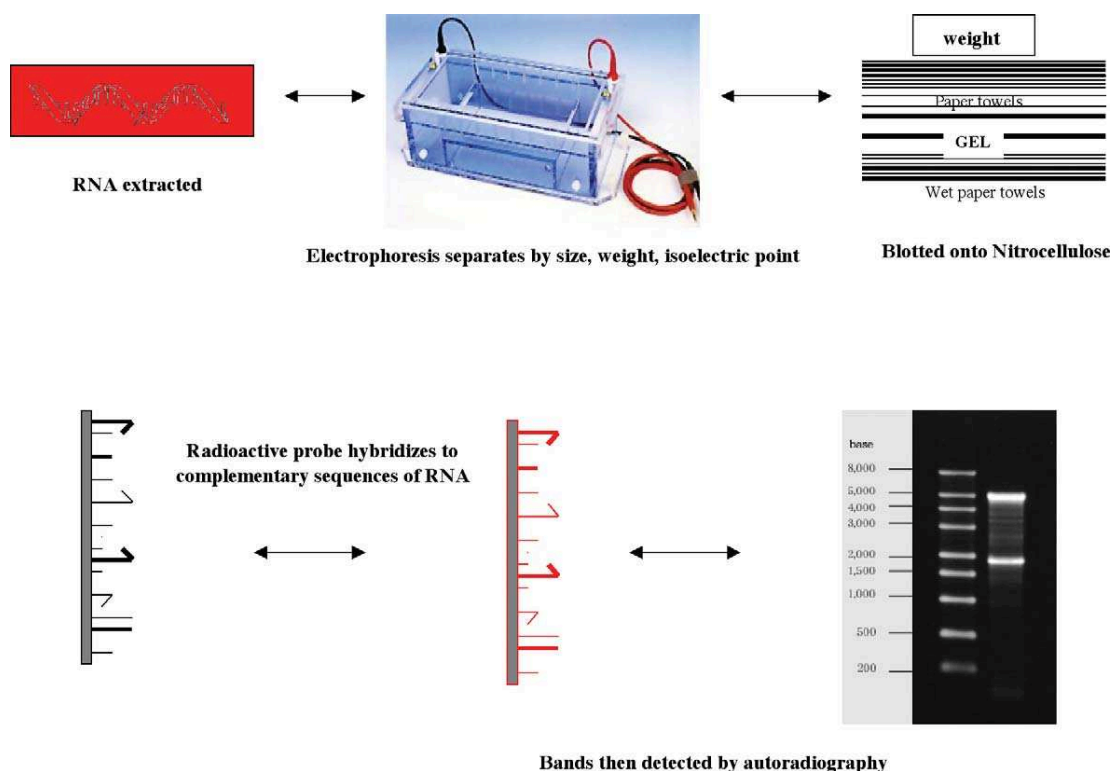
Figure 8-3

Northern blotting method box. RNA is extracted from cells or tissue and gel electrophoresis is used to separate mRNA molecules according to their physical characteristics (such as size, shape, or isoelectric point). Once separated, these molecules are blotted onto nitrocellulose paper, and a labeled probe, containing complementary DNA sequences to the RNA sequence in question, is applied to the blot and allowed to hybridize. The labeled cDNA is then detected by autoradiography, chemilluminescence or phosphoimaging.

mixture is then exposed to RNase enzymes that digest single RNA strands. The RNA-RNA duplex is "protected" from this digestion and can be identified by gel electrophoresis. RNA protection assays may be most useful in the detection of rare mRNAs. RNA protection assays are labor intensive and are rarely used in the clinical management of cancer patients. Selenomethione, an organic compound, is a known chemoprotective agent. Its identification as a modulator to cyclin B and Cdc2 kinase activity, hence its effect on cell cycle control, was discovered in a colon cancer cell line via expression measurements by RNase protection assays, proving that pathway analysis is possible utilizing this technique.[36]

RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) is one of the most sensitive techniques currently available for mRNA detection and quantification. RT-PCR can be used clinically to screen for the expression of low copy transcripts in several body fluids and tissues (Figure 8-1*b*).[37] The simplicity of PCR and RT-PCR has

resulted in its use in several clinical diagnostic and monitoring tests. A recent modification of the RT-PCR technique is referred to as real-time RT-PCR or quantitative RT-PCR. Real-time PCR provides not only detection of a mRNA transcript but also can define the relative amounts of mRNA found in a sample.

RT-PCR can quickly allow for precise quantification of mRNA levels within samples and has a number of defined clinical applications. It is commonly used in the identification of aberrant oncogenic fusion products. Bcl-2 translocations in follicular lymphoma and PML-RARα fusion transcripts in acute promyelocytic leukemia (APL) are commonly identified by this method.[38a,38b] RT-PCR is also used to identify molecular remission patients with APL. Molecular remissions are defined by the absence of not only clinically detectable disease but by the absence of any tumor-associated transcript in blood, bone marrow, or lymph node. Achieving a molecular remission in patients with leukemias and lymphoma is a superior measure of prognosis over clinical assessments of remission that are based on clinical examination, imaging techniques, or histological or cytological assessments of at-risk tissues.[39–42]
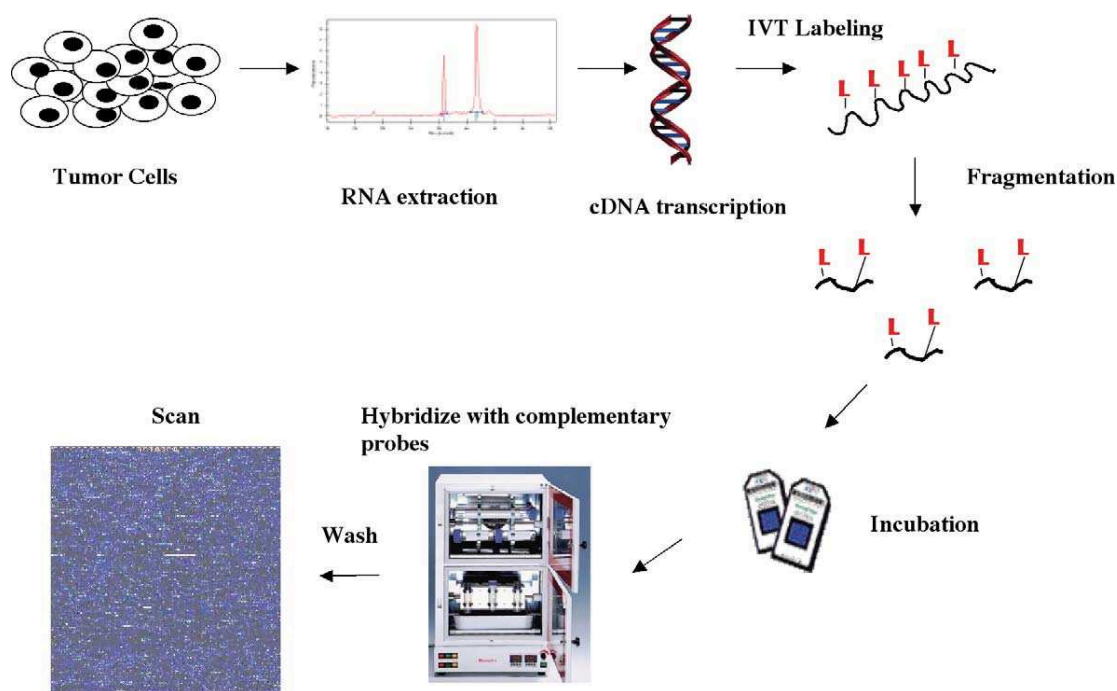
Figure 8-4

Expression microarray method box. Expression microarrays are a chip or microscopic slide on which cDNA or oligonucleotides have been printed or spotted. A number of different platforms can be utilized to develop an expression microarray. These include short oligonucleotide probes (25 bases or less), longer oligos (35 to 70 bases), or cDNAs. RNA from the cells or tissue in question is extracted and then fluorescently labeled through a number of methods defined by the specific microarray platform that is used (labeling may include cDNA or cRNA). The labeled cDNA or cRNA is incubated on the glass slide or chip to allow hybridization by complementarity probes printed on the microarray surface. Each distinct probe on the microarray is scanned for the intensity of fluorescence, which will be a reflection of the quantity of that probe in the original biological sample.

Expression Microarrays

Microarrays describe a new technology platform that allows the printing of DNA, cDNA, oligonucleotides, artificial chromosome segments, protein lysates, or antibodies on small glass slides (standard glass microscope slides) or chips (often the size of a 25 cent piece). The arrays are described based on the material that is printed on the slide or chip. In all cases, the array process allows the assessment of thousands of distinct elements in a single experiment (Figure 8-4). Expression microarrays are microarrays that define the expression of mRNAs in a tissue.[32,43] An expression array provides information on thousands of mRNAs that is similar to that provided by quantitative RT-PCR or Northern blot for a single mRNA.

Two distinct platforms are used for the expression microarrays. The original expression microarrays (referred to as two-color arrays) involve the labeling of two biological samples (for example, a primary tumor and metastasis) with two fluorescent labels (for example, red and green; pseudo colors). After labeling, the samples are mixed together before they are applied to the array for hybridization. The difference in gene expression between the two biological samples is quantified as the intensity of red to green signal at each printed cDNA. By using a common reference sample, the relative pattern of gene expression in multiple samples can be defined. This two-color approach is more commonly seen with cDNA microarrays where double-stranded cDNAs are printed onto the glass slides. A more recently developed platform involves the synthesis and then printing of single-stranded oligonucleotides to slides or chip surfaces. The actual sequences of oligonucleotides (usually more than one sequence is used for each gene) found in this array platform are based on bioinformatics algorithms that reduce both false-positive and false-negative hybridizations. The hybridization reaction for this type of array is most commonly a single-color approach where one biological sample is applied to each array. Comparisons between samples are analyzed after the hybridization and analysis of each sample using internal "housekeeping" controls on each array. The flexibility and reproducibility of using oligonucleotide arrays is a significant advantage for this array platform that comes with greater unit cost.

The data from expression microarray analysis can be used in a reductionist manner where an investigator can determine which individual genes are expressed in a biological sample and their relative level of expression. This approach can lead to the identification of new genes that may be associated with a specific disease or disease process, for example, the use of microarrays to identify genes associated with the metastatic phenotype of cancer.[44,45] Increasingly, microarray data have been used to define a pattern of gene expression (expression signature) for samples. This signature is often based on a subset of the entire microarray dataset. Although this signature is made up of distinct genes (or cDNAs), the pattern of expression of all genes in the list is more valuable than any single gene. This pattern can also be used to define signaling pathways associated with a disease process. Using this approach, Staudt et al. defined expression patterns that better define prognostic groups within otherwise indistinguishable human patients with diffuse large B-cell lymphoma.[46–48] Similar studies have defined expression patterns that are predictive of metastatic outcome in human patients with a variety of tumor types.[49] A number of novel bioinformatic approaches have been designed to assist in either the reductionist or global assessment of microarray data. Clustering analysis is created from these genetic signatures in which relationships between samples are defined (Figure 8-5).[50–55] The distance between data points, in cluster analyses, represents a distance in space, which encapsulates how similar two samples are in regard to their gene expression.
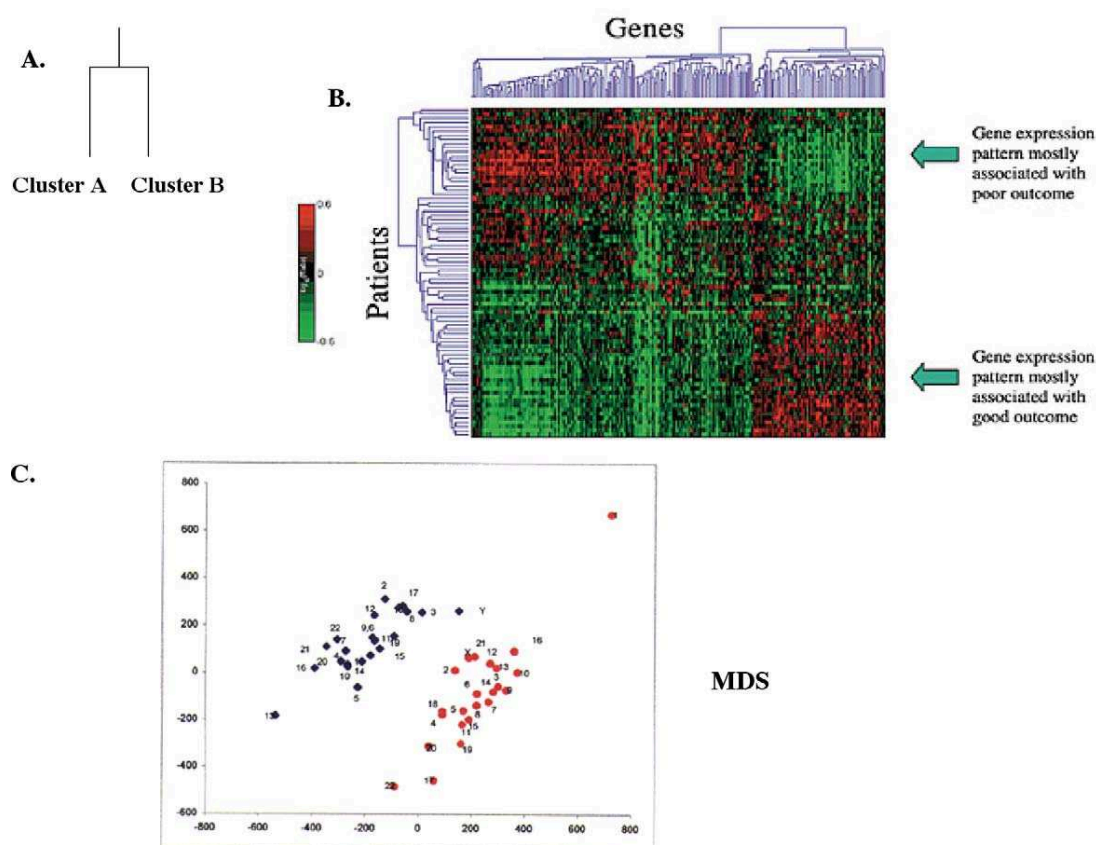
Figure 8-5

Clustering of microarray data using hierarchical clustering and MDS. **A,** Hierarchical clustering analysis is a statistical method for finding relatively homogeneous clusters of cases based on measured characteristics. For microarray data these characteristics are genes, or genetic relationships. This can be represented by a dendrogram or tree, where each step in the clustering process is illustrated by a join of the tree. **B,** Dendrograms can be used to illustrate the grouping of patients tumors based on a grouping of genes, like those above associated with prognosis. **C,** Multidimensional scaling (MDS) clustering of gene expression data displays the position of each tumor sample in a three-dimensional Euclidean space, with the distance between the samples reflecting their approximate degree of correlation. It is a spatial relationship that conveys in a three-dimensional form the relationships depicted in the dendrogram.

Unsupervised clustering includes all genes in the analysis and is the more traditional mode of evaluation. However, supervised clustering—in which certain gene groups are defined to guide the search—is also utilized. Ontology descriptors, describing cellular or molecular functions, have been applied to data sets from microarray studies. An unbiased assignment of functions to a gene list can allow definition of processes or pathways relevant to a cancer and have been used to rationally assist in drug development.[50-62]

A canine expression microarray is commercially available from Affymetrix (www.affymetrix.com). The utility of this and other privately held or privately developed canine microarrays is currently hindered by limited gene annotation for the dog. The release of the canine genome sequence draft, and its availability on large publicly accessible databases (Ensembl and NCBI) has significantly reduced this problem (www. ensembl.org and www.ncbi.nlm.nih.gov). Efforts are underway to define the expression profiles of a variety of canine tumors using expression microarrays. Results of these studies will contribute to our systems understanding of the biology of canine and eventually feline cancers.

Microarray analysis of gene expression has changed our understanding of cancer biology and has led to a more global and systems-based understanding of the mechanisms of cancer development and progression. Furthermore, the opportunity to compare the expression profiles of biological samples, across thousands of genes in a single experiment, has also defined novel targets for cancer therapy. The current state of the art has not yet provided the consistency of analysis between microarray platforms or across patient populations to allow global signatures of genes to be used as diagnostic tests. It is more likely that microarray data will provide informative genes or sets of messenger genes that may be later included on a multiplexed (quantitative) PCR platform and used as new diagnostic tests.

METHODS FOR PROTEIN ANALYSIS

Introduction

Protein expression represents the accumulation or end product of genetic information. DNA is transcribed into RNA, which is then translated into proteins. Therefore, the detection of proteins and their active forms (most often phosphorylated) represents an important step in the global understanding of cancer biology. Protein measurements can be both qualitative and quantitative and can include a variety of candidate approaches including Western blotting, immunohistochemistry and various novel noncandidate proteomic platforms, collectively referred to as proteomic approaches.[34,63]

Western Blots

Western blots allow for the detection of a protein within a sample through the use of an antibody that is specific to that protein (Figure 8-6). Western blots are used widely as a diagnostic modality that has recently seen expanded clinical use in the field of oncology through the availability of antibodies that are specific for phosphorylated forms of proteins and for mutated proteins. As treatment modalities for cancer have become more target-dependent, the need has increased to define the presence of the target protein in a clinical sample. Documentation of HER2/neu oncogene expression in human breast cancer patients has the dual utility of contributing to prognosis (expression of this protein carries a negative prognostic value), as well as guiding the rationale use of trastuzumab (Herceptin), a therapeutic monoclonal antibody that has been shown to improve time to progression and overall survival in metastatic breast cancer patients, when combined with chemotherapy.[64-67]

In the veterinary field, Western blots have been part of the diagnosis and management of animals with tick-borne infectious disease and feline immunodeficiency virus. The use of in-house "snap" test technology is based on a similar technique of antibody-based detection, including enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). In these assays, rather than using gel electrophoresis, the probing antibody is affixed to a substrate for detection of antigen in material passed over or through this substrate. As such, the specificity and sensitivity of antigen-antibody interactions needed for these tests are greater than what are needed for a Western blot.[14,68,69] In the field of oncology, these snap tests have been limited by a lack of tumor-specific antibodies (with high sensitivity and specificity) that could aid in the diagnosis of cancer.

Immunhistochemistry and Flow Cytometry

As in the case of Western blots, advances in antibody development have similarly improved the opportunity to define patient groups and direct therapy through the use of immunocytochemistry (assessment of protein expression in fixed cells), immunohistochemistry (assessment of protein expression in fixed tissue sections), or by fluorescent-activated cell sorting (FACS; the use of fluorescent-labeled antibodies to detect proteins in tumor lysates or body fluids, also known as flow cytometry).[14]

In veterinary oncology, immunocytochemistry/ histochemistry and FACS are used clinically to define immunophenotypic variation in canine lymphoma (CD3 positivity for T-cell disease and CD79a positivity for B-cell disease) and for dogs with mast cell
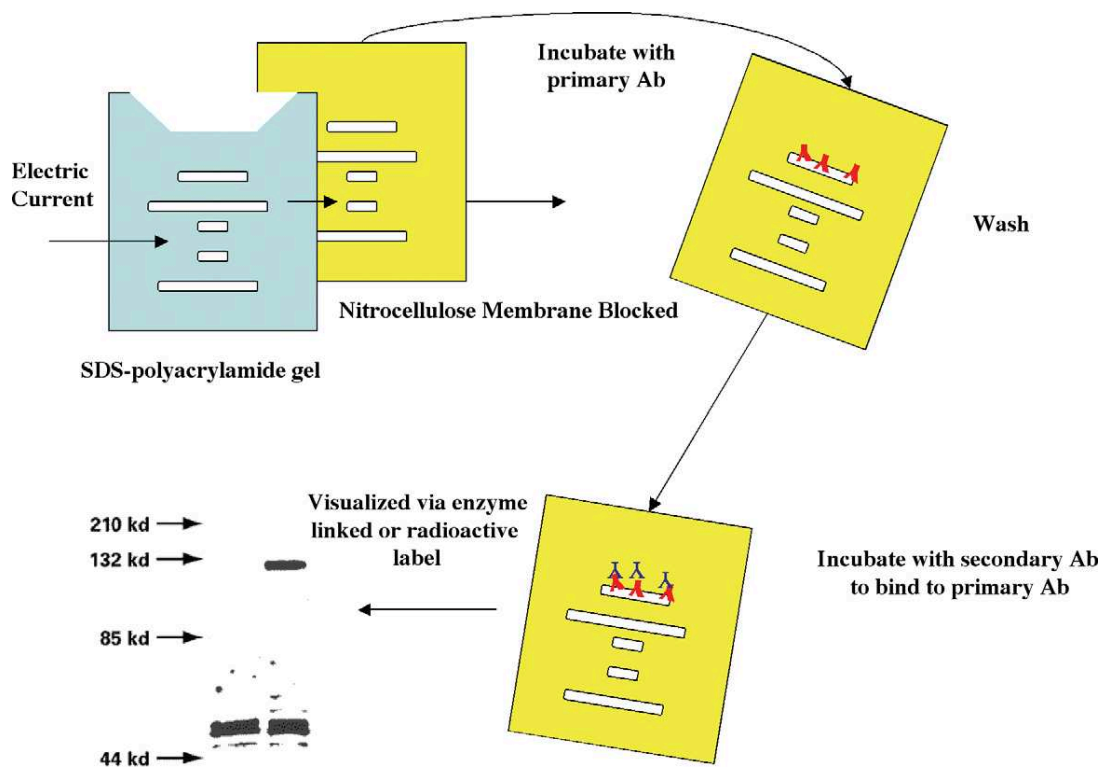
Figure 8-6

Western blotting method box. Like Southern and Northern blotting, gel electrophoresis is first used to separate proteins based on their size and charge using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). SDS is a detergent that lyses cells and denatures proteins before adding a negative charge. SDS addition allows proteins to migrate strictly based on length and removes the complexity that varying folding patterns would create for efficient migration. It also binds to protein in a constant ratio giving a uniform mass: charge ratio to all proteins, so the distance migrating relates to size of the protein alone. Denatured proteins are applied into the wells of a gel submerged in water. An electrical current is applied across the gel to initiate migration. After a set amount of time, the proteins in the gel are transferred onto a membrane made of nitrocellulose or PVDF by pressure or by application of a current. The "blot" refers to this process of transferred proteins. The membrane is then blocked to prevent nonspecific protein interactions between the membrane and soon-to-be-applied antibodies with a solution of bovine serum albumin (BSA) or dry milk. Without blocking, the antibody would bind nonspecifically to the nitrocellulose filter. A primary antibody is then incubated with the membrane; this recognizes only the protein of interest. After rinsing to remove unbound primary antibody, the second antibody incubation is performed. Its purpose is to bind to the first antibody. It is often linked with an enzyme or radioactive label for visualization.

tumors (c-kit; CD117).[70-73] The use of combinations of antibodies to positively and negatively select specific cells using FACS (referred to as multicolor FACS) has resulted in the ability to detect very specific populations of cells in peripheral blood. These cells have included circulating tumor cells or circulating endothelial cells. Single circulating cell detection has been used to define minimal residual disease in human patients with leukemias and solid tumors and has also been assessed in canine patients with lymphoid malignancies.[19,24,74-77] Circulating endothelial cells and subsets of activated and apoptotic endothelial cells in dogs treated with antiangiogenic agents has been described using multicolor FACS (personal communication, E. McKeegan). The increasing specificity of new antibodies, and the resilience of this specificity when used on formalin-fixed tissues, has contributed to the clinical value of these antibodies. The costs of development of conventional whole protein antibodies and peptide antibodies (raised against short segments of the target antigen) have been significantly reduced. As such, the availability and opportunity to generate new canine or feline-specific antibodies has been expanded. At the same time, a number of groups are actively examining the cross-reactivity of human antibodies for dog or cat tissues.

Proteomics

Just as microarrays made it possible to evaluate several genes within a biological sample in a single experiment, proteomics refers to the large-scale and high-throughput study of proteins, particularly their structure and function.[78–80] This relatively new field, similar to the use of genomics, is often viewed as the next step in the use of the systems biological approach to understanding cancer and other disease states. The proteome represents the spectrum of proteins expressed in a particular cell, tissue, or species. It is a dynamic entity that is much less stable and more complicated than the genome. Protein expression is tightly regulated and influenced by translation, protein activation (most often by phosphorylation) or other posttranslational modifications, and then protein turnover and degradation. Thus, protein expression in a disease state is not solely defined by its genetic expression pattern and can change quickly over time. One fascinating finding from the Human Genome Project is that there are many more proteins within the human organism than there are genes to code for them; specifically, approximately 33,000 genes have been identified in humans and more than 200,000 proteins.[79] This diversity must be due to the posttranslational modification of proteins or alternative splicing of many of these genes, leading to distinct translated proteins from a single gene. Several technological advances have allowed the rapid identification and characterization of proteins. These methods will be described briefly here.

Mass spectrometry (MS) is a technique for separating ions by their mass-to-charge ratio. Since the mass and charge of a protein is unique, MS can be used to identify proteins within a sample by comparing the MS results with established protein databases. Mass spectrometry is commonly employed in the measurement of protein expression in a variety of proteomic techniques including these described here.[25,79]

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is a technique used to separate proteins by both their isoelectric point (pI; in the first dimension) and by their mass (second dimension). A sample containing a protein mixture is optimized and solubilized for isoelectric focusing. The sample is applied to a first gel, where an electric current is used to separate proteins by pI. After further processing, this gel is applied to the top of a second gel (a traditional SDS-PAGE gel), where application of an electric current will force the migration of the proteins from the first to the second dimension gel, where the proteins will then be separated based on their mass. This technique allows the separation of proteins into distinct spots within a gel; gel plugs of these spots can be removed and further analyzed by mass spectrometry to determine the identity of each individual protein spot. Depending on the size of the gels used, some overlap of proteins may occur, but for the most part each spot will represent a single protein.

Surface-enhanced laser-desorption ionization (SELDI) is a protein profiling strategy that combines sample prefractionation with MS.[80] It allows for the study of low molecular weight proteins (<20 kD) that would be excluded by 2D-PAGE. The data obtained by SELDI experiments are also more easily translatable to a bioinformatics platform and thus can identify protein patterns and signatures that may be useful in the examination of patient samples. SELDI has been used to generate tumor-specific protein profiles and has great utility in the profiling of biologic fluids, such as urine and serum. Pattern recognition studies to identify profiles for breast and prostate cancers have approached 100% specificity with this method, which incorporates comparisons to normal controls. As with microarrays, proteomic techniques like SELDI can be used as discovery tools that may uncover potential targets for cancer diagnosis or drug therapy.[78,79,81]

CLINICAL APPLICATION OF NOVEL DIAGNOSTICS

As the field of oncology moves toward biology-based therapeutics, it will be increasingly important that parallel target-based molecular diagnostics are developed. This parallel development will lead to opportunities to tailor treatment options to specific and potentially "driving" elements (genes, proteins, or pathways) in a cancer. Diagnostic tests will be needed to define patients likely and potentially most likely to respond to a treatment, determine early after initiation of a therapy that a specific therapy is acting against the target, which may be assessed by actually evaluating the target or some associated measure (i.e., surrogate endpoint), and guide the ongoing use of a therapy in the event that the next generation of treatments can be given over long periods of time without the risks of the typical toxicities of current anticancer agents. The duration of therapy may be best assessed by diagnostic tests that can determine the status of a response (or remission) at the molecular level.

LOOKING AHEAD: CASE STUDY OF CANINE LYMPHOMA

The traditional methods of diagnosis for canine lymphoma have been cytologic or histopathologic examination. Often these methodologies are sufficient for diagnosis of this common canine neoplasm, but occasionally they fall short or are confounded by other challenging clinical scenarios. A clinical example will be used to illustrate the molecular techniques presently available in veterinary oncology.

A 6-year-old female, spayed golden retriever presented to a veterinary referral hospital with a 1-week history of lethargy. Upon physical examination, mild, firm, and nonpainful generalized lymphadenopathy was palpable and the rest of the exam was unremarkable. Fine needle aspiration of the submandibular and popliteal lymph nodes was performed, and a homogeneous population of medium-sized lymphocytes was described on clinical pathologic review. A CBC, chemistry profile, urinalysis, and thoracic radiographs were obtained revealing a moderate lymphocytosis (12,000 medium-sized lymphocytes) and pronounced thrombocytopenia (40,000 platelets). A diagnosis of lymphoma was suspected and a submandibular lymph node was removed for histopathologic examination. A bone marrow aspirate was also obtained from the proximal aspect of the right humerus, revealing sheets of medium-sized lymphocytes and megakaryocytic hypoplasia.

A histological evaluation of the lymph node biopsy was inconclusive, as sheets of medium-sized lymphocytes were seen but lymph node architecture remained intact. Lymphoma was still suspected, and as tick titers were procured, a second fine needle aspiration of a representative enlarged lymph node was completed. The aspirate was placed into a red top tube for PCR assessment of clonality and part of the bone marrow aspirate was submitted for flow cytometry.

Clonality is the hallmark of malignancy. PCR is able to detect the clonal rearrangement of the antigen receptor immunoglobulin or T-cell receptor genes (see Figure 8-2). The sensitivity of PCR in the diagnosis or canine lymphoma is 91%.[82] PCR assessment of lymphoid tissue, bone marrow, or peripheral blood is possible and facilitates the diagnosis of lymphoma in these puzzling clinical scenarios. This methodology can also be used to detect minimal residual disease (MRD) in lymphoid malignancies, possibly identifying patients who require further therapy. PCR detection of MRD is known as molecular positivity. PCR is commercially available in only a small number of veterinary laboratories. About 1 week after submission, PCR results revealed B-cell lymphoid clonality. FACS analysis on the bone marrow aspirate revealed that the neoplastic cells were CD21+, CD3−, and CD34+. This confirmed a diagnosis of B-cell acute lymphoblastic leukemia (ALL). CD34 is a glycosylated glycoprotein, which is expressed on early lympho-hematopoietic stem and progenitor cells, capillary endothelial cells, and bone marrow stromal cells. CD34 expression is useful in the diagnosis of lymphoid or myeloid leukemias but is not seen in lymphoma or chronic lymphocytic leukemia. Its presence represents an early phase of differentiation.

This level of molecular diagnosis is available in clinical practice and is sufficient to provide an appropriate diagnosis of ALL and lead to the development of an aggressive treatment regimen of CHOP-based chemotherapy for this patient (see Chapter 31, Section A). As many of the diagnostic methods discussed in the previous chapter advance to the clinic, it is likely that additional clinical data will inform the treatment and management of this patient. It may be possible to use microarrays to determine the prognosis of this specific patient. Similar analyses are now possible in the management of human patients with ALL. If a subset of ALL patients could be identified that have a more favorable prognosis, it is possible that the owners of these dogs would be more comfortable with an aggressive treatment approach. The use of microarrays may define ALL as a cancer expressing a unique target for therapy. Such targets have been identified in a form of anaplastic large cell lymphoma in human patients. When this target, called ALK, is expressed, the response rate to a novel small molecule inhibitor, Staurosporine has been significant.[83–89] Once an appropriate therapeutic approach, based on conventional cytotoxic chemotherapy, and novel targeted therapy are defined, it may be possible to follow a surrogate marker of response to determine if changes in approach are necessary. The surrogate markers may be expressed in blood, urine, or saliva and may include a profile of small proteins identified by proteomic approaches or a target cell identified and quantified using multicolor FACS. It is likely that a molecular-based (PCR) assay for tumor will then be used to determine how long therapy should be continued and to then monitor the dog for early relapse.

It is likely that the use of molecular diagnostics for cancer will become more widespread and more integrated not only in the initial diagnosis of patients but also into the management of dogs and cats with malignant disease.

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Biopsy Principles

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BIOPSY PRINCIPLES

A biopsy refers to a procedure that involves obtaining tissue for microscopic or other analysis to establish a precise diagnosis or enhance the understanding of a particular tissue with regard to histologic, molecular, phenotypic, etiologic, or immunohistochemical characteristics. Histopathologic interpretation of tissue removed from a tumor is not foolproof and is highly dependent on the quality and quantity of the biopsy sample submitted. It is important to understand basic principles of biopsy procurement and submission in order to obtain an accurate diagnosis. If the tissue diagnosis is incorrect, every subsequent step in the treatment of the patient will often be incorrect.

Several techniques are available for obtaining tissue specimens ranging from needle-core techniques to complete excision. The choice of technique depends on the anatomic location of the tumor, the patient's overall health, the suspected tumor type, the analysis to be performed, and the clinician's preference. Biopsy techniques can be grouped under one of two major categories: pretreatment biopsy (needle core biopsy, punch biopsy, wedge biopsy, etc.) or excisional biopsy. Pretreatment biopsy is performed to obtain additional information about the tumor prior to definitive treatment. Posttreatment (i.e., excisional) biopsy refers to the process of obtaining histopathologic information following surgical removal of the tumor. Excisional biopsy is best used to obtain a more complete picture of the disease process (tumor grade, histologic subtype, percentage of necrosis, degree of invasion into regional vasculature and lymphatics, etc.) and provides an opportunity to evaluate completeness of excision. It is rarely ever the best first step in obtaining a tissue diagnosis. While excisional biopsy is attractive to many clinicians because it allows for definitive treatment and diagnosis in one step, it is often used inappropriately in the management of a cancer patient, potentially resulting in incomplete surgical margins. Incomplete surgical margins result in local recurrence, the need for additional adjuvant therapy (e.g., radiation or chemotherapy), or the need for a wider, more extensive surgery. All of these sequelae represent compromise of the optimum treatment pathway for the patient and may involve more morbidity and expense than a properly performed first excision. The issue to be determined before surgery then is how aggressive the surgery to remove the tumor should be. It is intuitive that wide, ablative surgery (e.g., body wall resection) would be inappropriate for a simple lipoma. It also follows that marginal excision ("shell out") is inappropriate for definitive treatment of an aggressive tumor such as a soft tissue sarcoma. Thorough knowledge of the tumor type and suspect biology is imperative prior to attempting surgical excision. The best way to obtain this information is often via pretreatment biopsy.

Specific indications for pretreatment biopsy are as follows:
1. When the *type* of recommended treatment (radiation versus chemotherapy versus surgery) would be altered by knowledge of the tumor type.
2. When the *extent* of recommended treatment (ablative surgery versus wide excision versus marginal excision) would be altered by knowledge of the tumor type.
3. When the tumor is in a *difficult area to reconstruct* (maxillectomy, locations requiring extensive flaps, head and neck) and planning is needed to prepare the patient and client appropriately.
4. When knowledge of the tumor type and its likely biological course (i.e., prognosis) would *change the owners willingness to go forward* with curative-intent treatment.

There are occasions when pretreatment biopsy would be contraindicated. These include cases where the type of treatment or extent of surgery would not be changed by knowing the tumor type (e.g., testicular mass, solitary splenic mass) or when the surgical procedure to obtain the biopsy is as risky as definitive removal (e.g., spinal cord biopsy). In these cases, the patient would best be

served by excisional biopsy of the tumor if staging results support this choice.

BIOPSY METHODS

The more commonly used methods of tissue procurement are needle core biopsy, punch biopsy, incisional (wedge) biopsy, and excisional biopsy.

Needle Core Biopsy

Needle core biopsy utilizes various types of needle core instruments to obtain soft tissue[1,2] (Figure 9-1). Most of these needles are manually operated, although spring and pneumatically powered needles are available as well. Specialized needle core instruments are used for bone biopsies and will be covered in Chapter 23.
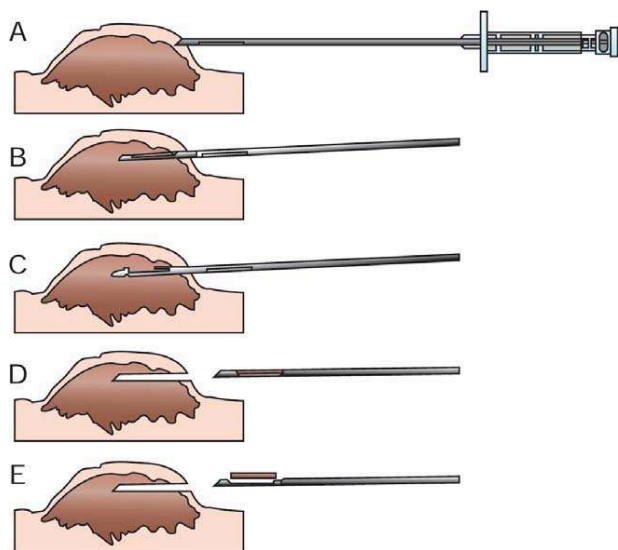
Figure 9-1

Mechanism of action of needle core biopsy needle for typical nodular tumor. **A,** A small skin incision is made with a Number 11 blade to allow insertion of the instrument. With the instrument closed, the outer capsule is penetrated. **B,** The outer cannula is fixed in place and the inner cannula with the specimen notch is thrust into the tumor. The tissue then protrudes into the notch. **C,** The inner cannula is now held steady while the outer cannula is moved forward to cut off the biopsy specimen. **D,** The entire instrument is removed closed with the tissue contained within it. **E,** The inner cannula is pushed ahead to expose the tissue in the specimen notch.

Clinicians may find it helpful to practice the use of needle core instruments on apples prior to biopsy of an actual tumor. These instruments are generally 14-gauge in diameter and procure a piece of tissue that is about 1 mm wide and 1.0 to 1.5 cm long. In spite of this small sample size, the structural relationship of the tissue and tumor cells can usually be visualized by the pathologist. Virtually any accessible mass can be sampled by this method. It may be used for externally located lesions or for deeply seated lesions (kidney, liver, prostate, etc.) with image-guidance via closed methods or at the time of open surgery.

The most common usage of the needle core biopsy is for externally palpable masses. Except for highly inflamed and necrotic cancers (especially in the oral cavity), where incisional biopsy is preferred, most biopsies can be done on an outpatient basis with local anesthesia or sedation. The area to be biopsied is clipped and cleaned. The skin or overlying tissue is prepared as for minor surgery. If the overlying tissue (usually skin and muscle) is intact, it is blocked with local anesthetic in the region that the biopsy needle will penetrate. Tumor tissue itself is poorly innervated and generally does not require local anesthesia.

The mass is fixed in place with one hand or by an assistant. A small 1- to 2-mm stab incision is made in the overlying skin with a scalpel blade to allow insertion of the biopsy instrument. Through the same skin hole, several needle cores are removed from different sites to get a "cross section" of tissue types within the mass. The tissue is then gently removed from the instrument with a scalpel blade or hypodermic needle and placed in formalin. For smaller-gauge needles, the tissue may be flushed off the needle with saline. Samples may be gently rolled on a glass slide for cytologic preparations before fixation. With experience, the operator can generally tell from the appearance of the core sample whether diagnostic material has been attained. Samples that float on the surface of formalin are generally composed of fat, and if not consistent with the clinical picture imply the sample is inadequate. Clients should be educated prior to the performance of any incisional biopsies technique that not all biopsies result in a diagnostic specimen and that a subsequent biopsy procedure may be necessary in some cases. Soft tissue sarcomas in particular may not yield good tissue cores because of necrosis and fibrous septa that often permeate the mass. Sutures are generally not required in the skin hole. Needle biopsy tracts are probably a minimal risk for local tumor seeding but if possible are removed intact with the tumor at subsequent resection.

Many of these needles are disposable with plastic casings and, therefore, cannot be steam sterilized. They may, however, be gas sterilized (with ethylene oxide or hydrogen peroxide gas) and used repeatedly until they become dull. Needle core biopsies are fast, safe, easy,

cheap, and usually can be performed as outpatient procedures. They are generally more accurate than cytology but not as accurate as incisional or excisional biopsy. A comparison of needle core biopsy and surgical biopsy for skin or subcutaneous masses revealed a 96% agreement rate when comparing both techniques on the same lesion. It must be noted that this was a study by a single institution and a single pathologist and that the accuracy and correlation rate would undoubtedly decrease somewhat with different pathologists. It needs to be remembered that for a 5- by 5-cm mass, one core biopsy probably represents less than 1% of the tumor tissue.

Punch Biopsy

Punch biopsy tools were originally designed for biopsy of the skin (Figure 9-2). They deliver a shorter and wider (2- to 6-mm) biopsy than does a needle core. They can be used on any external, relatively flat tumor (skin, oral, perianal). They do not work as well for tumors located under intact skin unless the skin is first incised. Preparation of the site is the same for core

biopsies. Once the punch has cut into the tumor, the core is gently lifted and the base of the core is cut off with scissors. One or two sutures may be placed if intact skin or mucous membrane were opened.

Incisional Biopsy

Incisional biopsy is utilized when neither cytology nor needle core biopsy has yielded diagnostic material (Figure 9-3). Additionally, it is preferred for ulcerated and necrotic lesions, since more tissue can be obtained. Unfortunately, many cancers are large, exophytic, and ulcerated. These tumors are poorly innervated and may be biopsied without the need for local anesthesia or sedation. Under "sterile" conditions, a wedge of viable tumor tissue is removed from the mass. Ideally, a composite biopsy of normal and abnormal tissue is obtained, provided it will not compromise subsequent curative resection or contaminate uninvolved tissue needed for reconstruction. Care should be taken not to widely open uninvolved tissue planes that could become contaminated with released tumor cells. Small incisions through expendable muscle bellies are preferred to
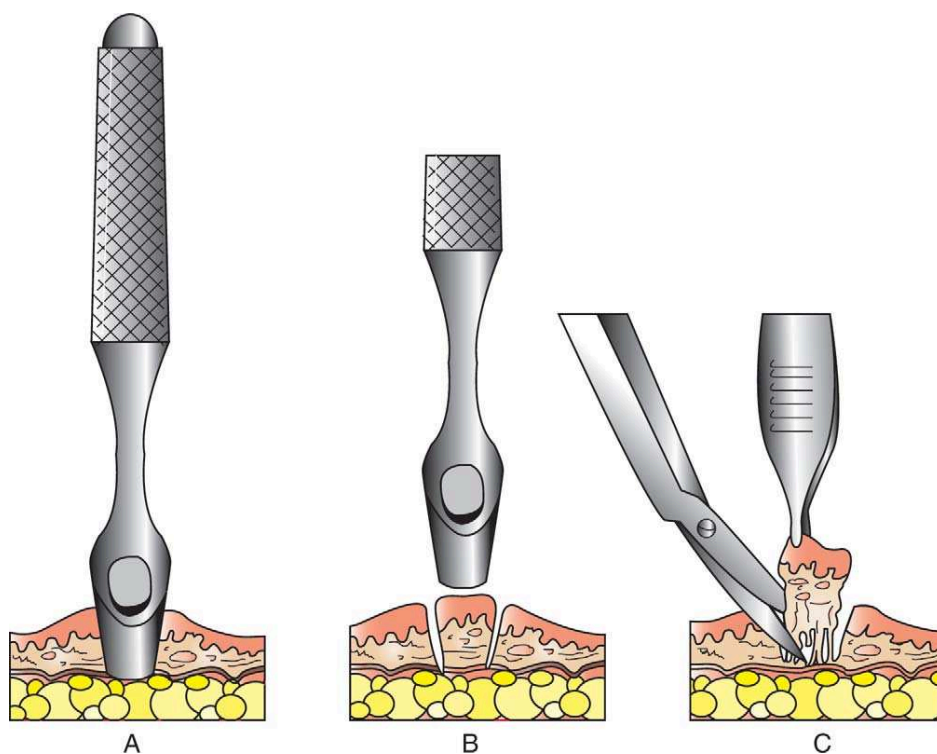
Figure 9-2

Mechanism of punch biopsy. **A,** Punch is rotated back and forth over suspect lesion until sufficient depth has been attained. **B,** Punch is removed or angled across base to sever deep attachments. **C,** Specimen may be "gently" grasped with thumb forceps and cut off deeply.
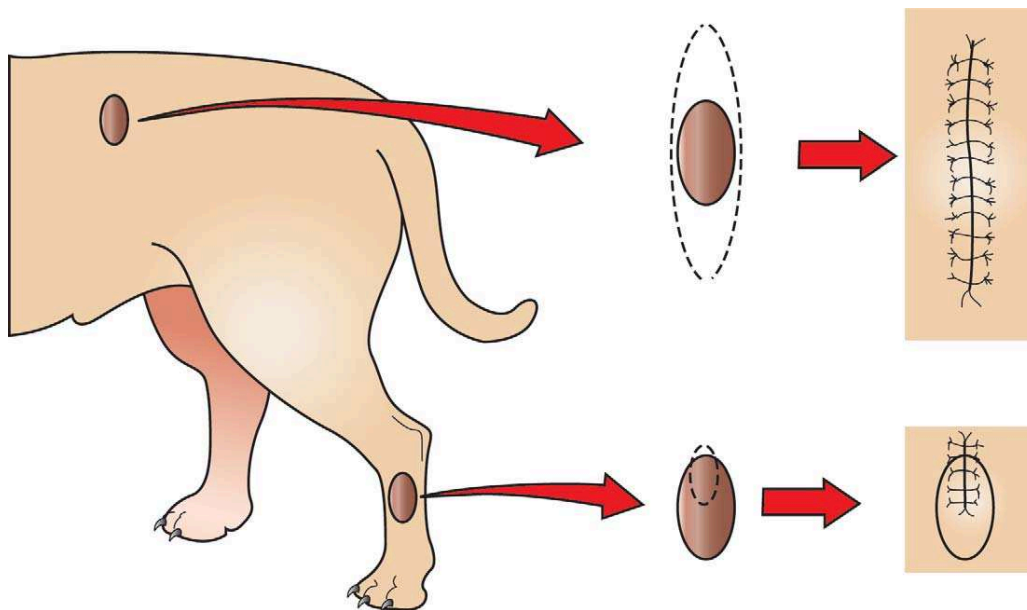
Figure 9-3

Excisional *(top)* contrasted with incisional *(bottom)* biopsy. The top tumor may be as easy to remove as to biopsy, and removal may not negatively influence other possible treatments (more surgery, radiation, etc.). The bottom tumor, however, requires knowledge of the tumor type prior to excision, because inappropriate removal could compromise a subsequent aggressive excision (short of amputation). Note that the biopsy incision is in a plane that would be included in a subsequent resection.

contaminating intramuscular compartments. The incisional biopsy tract is always removed in continuity with the tumor at subsequent resection.

Specialized Biopsy Techniques

Specialized biopsy techniques will generally be covered in chapters discussing the specific individual tumors. However, some general comments follow.

Endoscopic Biopsies

These techniques use flexible or occasionally rigid scopes that allow visualized or blind biopsy of hollow lumens, especially gastrointestinal, respiratory, and urogenital systems. Although these techniques are convenient, cost effective, and generally safe, they sometimes suffer from inadequate visualization and limited biopsy sample size when compared to other techniques. An endoscopic biopsy result of ulcerative gastritis in a dog with a firm, infiltrative mass of the lesser curvature of the stomach does not rule out gastric adenocarcinoma.

Laparoscopy and Thoracoscopy

Laparoscopic and thoracoscopic biopsy and surgery techniques are commonplace in human medicine and

are gaining popularity in veterinary medicine as well. Evaluation of the abdomen and thorax, when performed by an experienced operator, can yield important information regarding stage of disease and can procure tissue for biopsy. The drawbacks of these methods are that they can take as long as an exploratory laparotomy or thoracotomy (both requiring general anesthesia), they do not give the operator as good a visualization as open exploratory, they may not provide for excision, they carry some risk of hemorrhage or leakage of fluid (bile, urine, tumor cells, or bowel content), and tissue samples tend to be small. The best indication for these methods is when all staging procedures suggest diffuse and inoperable disease. Animals staged by whatever means as having solitary and potentially resectable disease are often best served by open exploratory laparotomy or thoracotomy whereby more definitive staging and hopefully curative-intent resection can be performed.

Image-Guided Biopsy

Diagnostic imaging has greatly expanded the ability to stage various neoplasias and even suggest possible tumor types. In addition, the use of radiographic, fluoroscopic, ultrasonographic, computed tomographic, and magnetic resonance image-guided needle aspirates or core biopsies

can avert the need for more invasive diagnostic proce-dures. Commonly biopsied tissue includes lung, kidney, liver, spleen, prostate, and, more recently, brain (see Chapter 29). All of the limitations of laparoscopy generally apply, and a careful consideration of the pros and cons of closed guided biopsy should be considered.

"Solitary" lung masses, much like solitary abdominal masses, are best diagnosed and treated by exploratory surgery. Lung masses localizable and adjacent or adherent to the thoracic wall can be safely aspirated or needle core biopsied with or without imaging and can potentially be excised by an experienced thoracoscopist. Transgressing normal lung, pleura, and tumor capsule can result in problems if subsequent surgical resection is contemplated. These "closed" biopsies are generally considered for animals with diffuse inoperable disease (e.g., lymphoma) in hopes that the diagnosis may help guide selection of chemotherapy.

Excisional Biopsy

This method is utilized when the treatment would not be altered by knowledge of tumor type ("benign" skin tumors, solitary lung mass, solitary splenic mass, retained testicular tumor, etc.). It is more frequently performed than indicated, but when used on properly selected cases, it can be both diagnostic and therapeutic as well as cost effective.

General Guidelines for Tissue Procurement and Fixation

1. When properly performed, pretreatment biopsies will *not* negatively influence survival, even though a short-lived increase in cancer cells can be measured in draining vessels and lymphatics. The advantages of an accurate diagnosis far outweigh the theoretical disadvantage of increasing the number of cancer cells temporarily in circulation. On the other hand, cancer cells may be allowed to contaminate the tissues surrounding the mass, making resection more difficult.[3] Careful hemostasis, obliteration of dead space, and avoidance of seromas or hematomas will minimize local contamination of the incisional biopsy site. Surgical drains should not be placed in biopsy sites if possible, because the drain tract can become contaminated with tumor cells. Furthermore, the biopsy site should be planned so that it may be subsequently removed along with the entire mass. In particular, care should be taken not to "spill" cancer cells within the thoracic or abdominal cavities during biopsy where they may seed pleural or peritoneal surfaces.
2. When biopsies are performed on the legs or the tail, the incision should be longitudinal and not transverse because transverse incisions are much harder to completely resect.
3. The junction of normal and abnormal tissue is frequently the best area for the pathologist to see differences in tissue as well as determine degree of invasiveness. Care should be taken, however, not to incise normal tissue that cannot be resected or would be used in reconstructing the surgical defect. Avoid biopsies that contain only ulcerated or inflamed tissues.
4. The larger the sample, the more likely it is to be diagnostic. Tumors are not homogeneous and usually contain areas of necrosis, inflammation, and reactive tissue. Several samples procured from one mass (preferably through a solitary entry site) are more likely to yield an accurate diagnosis than a single sample.
5. Biopsies should not be obtained with electrocautery or surgical lasers, as they tend to deform (autolysis or polarization) the cellular architecture. Electrocautery is better utilized for hemostasis after blade removal of a diagnostic specimen.
6. Care should be taken not to unduly deform the specimen with forceps, suction, or other handling methods prior to fixation.
7. Intraoperative diagnosis of disease by frozen sections, although not routinely available in veterinary medicine, has enjoyed widespread use in human hospitals. Special equipment and training are required for this technique to be fully utilized. One study in veterinary medicine revealed an accurate and specific diagnosis rate of 83%.[4]
8. If evaluation of margins of excision are desired, it is best if the surgeon marks the specimen (fine suture on questionable edges) or submits margins in a separate container. An alternate method of orienting the pathologist is the use of India ink in acetone or Alcian blue.[5] The resected tissue should be blotted with a paper towel, since the dyes will adhere better to the tissue. The tissue ink is "painted" on the surgical margins using a cotton swab. The dye should then be allowed to dry for up to 20 minutes before the tissue is placed in formalin. Tissue already fixed in formalin can be marked, but the dyes may not adhere as well and drying time is extended. When the pathologist reads the slides and observes tumor cells extending to the inked edge, the excision is incomplete.[6,7] Different-colored ink can also be used to denote different sites on the tumor, such as proximal margin or deep margin near nerve. Regardless of inking, proper fixation, and processing, the clinician must realize that two slides of a 2-cm

mass look at 0.5% of the surface, and it is estimated that to see every cell on the margin of a 2-cm mass, more than 3000 slides would be needed.[8] *As a general rule, a clinician can believe a diagnosis of an incomplete excision but not always that of a "complete" excision.*

It is vital that both the pathologist and the clinician communicate well what is required for accurate assessment of margins (see Chapter 3). Of course, margin evaluation is only necessary for excisional biopsy or curative-intent surgery and does not apply to needle core biopsies or incisional biopsies, which by definition will have inadequate margins. Incomplete surgical resection of malignant disease is best detected early so that further surgery or other effective adjuvant therapies can be instituted immediately, as opposed to waiting for inevitable local recurrence or metastasis.

9. If you have stainless-steel vascular clips in the resected specimen, it is wise to remove them before the tissue is submitted, because they can damage valuable microtomes.

10. Proper fixation is vital. Tissue is generally fixed in 10% buffered neutral formalin with 1 part tissue to 10 parts fixative. If more than one lesion has been biopsied, they should each be placed in a separate container.[5] As the technology rapidly expands in the general area of pathology and related fields, the need for special studies on tissue will increase. The clinician may want to consult with the pathologist on how to submit tissue for electron microscopy, hormone receptors status, tissue culture, flow cytometry, immunohistochemistry, or cytogenetics.

11. Tissue should not be thicker than 1 cm or it will not fix deeply. Large masses can be sliced like a loaf of bread, leaving the deep inked margin intact, to allow fixation. A commonly asked question is how to submit large visceral and especially splenic masses when 90% of the mass may be hematoma and only 10% neoplastic. Ideally the entire spleen is submitted, but an alternate approach is to have the surgeon take representative smaller samples from the mass (soft and hard pieces, red and pale pieces, top and bottom pieces, etc.) in the hope that one of them is diagnostic. The rest of the mass can be saved in the clinic in case more tissue needs to be evaluated. This extra tissue should be refrigerated and not frozen. Freezing causes severe artifact in the tissue. After fixation (2 to 3 days), tissue can be mailed with a 1:1 ratio of tissue to formalin or even wrapped in formalin-soaked paper towels and mailed in sealed plastic bags.

12. A *detailed* history should accompany all biopsy requests! Interpretation of surgical biopsies is a combination of art and science. Without all the vital diagnostic information (signalment, history of recurrences, invasion into bone, rate of growth, treatments used and result, presence of paraneoplastic syndromes, etc.), the pathologist will be significantly compromised in her or his ability to deliver accurate and clinically useful information.

13. A veterinary-trained pathologist is preferred over a pathologist trained in human diseases. Although many cancers are histologically similar across species lines, enough differences exist to result in interpretive errors.

Interpretation of Results

The pathologist's job is to determine (1) tumor versus no tumor, (2) benign versus malignant, (3) histologic type, (4) grade (if a clinically relevant grading system exists for that particular tumor type), and (5) margins (if excisional). Making an accurate diagnosis is not as simple as putting a piece of tissue in formalin and waiting for results. Many pitfalls can take place to render the end result inaccurate. Potential errors can take place at any level of diagnosis, and it is up to the clinician in charge of the case to interpret the full meaning of the biopsy result. As high as 5% to 10% of biopsy results are inaccurate in a clinically significant sense.[9] If the biopsy result does not correlate with the clinical scenario, several options are possible:

1. Call the pathologist and express your concern over the biopsy result. This exchange of information should be helpful for both parties and not looked upon as an affront to the pathologist's authority or expertise. It may lead to the following:
   a. resectioning of available tissue or paraffin blocks;
   b. special stains for certain possible tumor types (e.g., toluidine blue for mast cells);
   c. a second opinion by another pathologist or group of pathologists.
   In one study, interinstitutional review resulted in a change in diagnosis in 5.8% of cases reviewed.[10]

2. If the tumor is still present in the patient, particularly if widely varied options exist for therapy, a second (or third) biopsy should be performed.

A carefully performed, submitted, and interpreted biopsy may be the most important step in management and subsequent prognosis of the patient with cancer. All too often tumors are not submitted for histologic evaluation after removal because "the owner didn't want to pay for it." Biopsies should not be an elective

owner decision. Instead, they should be as automatic as closing the skin after ovariohysterectomy (Do you okay that with the owner?). The charge for submission and interpretation of the biopsy should be included in the surgery fee if need be, but the biopsy must be done. Because of increasing medicolegal concerns, it is not medical curiosity alone that mandates knowledge of tumor type.

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Surgical Oncology

Stephen J. Withrow

Complete surgical removal of localized cancer cures more cancer patients than any other form of treatment,[1] in part because this modality is generally applied as sole treatment for local, early stage disease, or tumors with limited potential to metastasize. Before this hope for cure can be realized, surgeons must have a thorough understanding of anatomy, physiology, resection, and reconstruction options for all organs, expected tumor behavior, and the various alternatives or adjuvants to surgery. Surgical oncologists should not only be good technicians (cancer carpenters), but dedicated tumor biologists. Surgery will play a role at one point or another in the management of most cancer patients. This utility of surgery may include any of the following: diagnosis (biopsy), resection for cure, palliation of symptoms, debulking (tumor cell cytoreduction), and a wide variety of ancillary procedures to enhance and complement other forms of treatment.

Surgical resection of cancer was introduced in the 16th century B.C. and remained relatively underutilized until general anesthesia (1840s), antisepsis (1860s), and pain management made aggressive resection safe and tolerable for the patient. Dr. William Halstead developed the basic principles of surgical oncology in the 1890s. The radical resection of the 20th century has been customized to meet the needs of the patient and has frequently been reduced in magnitude. Further refinements in surgery have been made possible with newer equipment (staples, endoscopy, etc.), advanced imaging, and critical care services.

Most patients with cancer are "old." "Old" is a relative term. It is much more important to know the physiologic age of the patient than its chronologic age. An "old" dog or cat with normal measurable organ function should not be denied treatment simply on the basis of age. I am aware of no cancer in which the older age of the patient has any direct bearing on tumor-related prognosis. In fact, dogs with osteosarcoma that are less than 2 years of age do worse than dogs that are more than 2 years of age after amputation alone.[2] "Old" animals, in most instances, will tolerate aggressive surgical intervention as well or as poorly as "young" patients.

SURGERY FOR DIAGNOSIS

Although biopsy principles are covered in Chapter 9, it bears emphasizing that properly timed, performed, and interpreted biopsies are one of the most crucial steps in the management of the cancer patient. Not only does the surgeon need to procure adequate and representative tissue to establish a diagnosis, additionally, the biopsy must not compromise subsequent curative surgical resection.

SURGERY FOR CURE

Before a surgeon can be in a position to provide the optimal operation for the patient with cancer, the following questions need to be answered:
1. What is the type, stage, and grade of cancer to be treated?
2. What are the expected local and systemic effects of this tumor type, grade, and stage?
3. Is a cure possible and at what price in terms of cosmetics and function?
4. Is an operation indicated at all?
5. What are the options for alternative or complementary treatment?

A recurring theme in surgical management of cancer is that the first surgery has the best chance of cure. Several mechanisms for this improvement in survival have been advanced. Untreated tumors have had less chronologic time to metastasize than recurrent cancer. Untreated tumors have near normal anatomy, which will facilitate operative maneuvers. Recurrent tumors may have had seeding of previously noninvolved tissue planes, requiring wider resection than would have been required on the initial tumor. If one thinks about a given cancer as resembling a crab, incomplete surgery removes the body of the crab and leaves the legs behind. The "body" of most tumors is often quiescent and hypoxic, whereas the leading edge of the tumor (legs) is the most invasive and well vascularized. Subtotal removal may actually selectively leave behind

TABLE 10-1	Classification and Resection of Wound Margins	
Type	**Plane of Dissection**	**Result**
Intracapsular	Tumor removed in pieces or curetted; "debulking"	Macroscopic disease left behind
Marginal	Removal just outside or on pseudocapsule or reactive capsule, "shelled out"	Usually leaves microscopic disease
Wide	Tumor and capsule never entered, normal tissue surrounds specimen	Possible skip lesions
Radical	Entire compartment or structure removed (e.g., amputation)	No local residual cancer

the most aggressive components of the tumor. Patients with recurrent cancer will often have less normal tissue for closure. An ill-defined negative aspect of recurrent cancer is reported to be related to changes in vascularity and local immune responses. Regardless of the mechanism, curative-intent surgery is best performed at the first operation.

The actual surgical technique will vary with the site, size, and stage of the tumor. Some general statements that need to be emphasized with surgical oncology are the following:

1. All incisional biopsy tracts should be excised in continuity with the primary tumor, since tumor cells are capable of growth in these wounds. Fine-needle aspiration cytology tracts are of minor, but not zero, concern, while punch biopsy tracts are of intermediate concern.[3] With this in mind, all biopsies should be positioned in such a manner that they can be removed at surgery.

2. Early vascular ligation (especially venous) should be attempted to diminish release of large tumor *emboli* into the systemic circulation. This is probably only clinically meaningful for those tumors with a well-defined venous supply, such as splenic tumors, retained testicles, and lung tumors. Small numbers of cancer cells are constantly being released into the venous circulation by most tumors. Larger, macroscopic cell aggregates may be more dangerous, however, and these may be prevented from vascular escape with early venous ligation.

3. Local control of malignant cancer requires that variable margins of normal tissue be removed around the tumor. Resection of the "bad from the good" can and should be classified in more detail than radical versus conservative (Table 10-1).[4] Tumors with high probability of local recurrence (soft tissue sarcoma, high-grade mast cell tumors, feline mammary adenocarcinoma, etc.) should have 2- to 3-cm margins removed in three dimensions. Tumors are not flat, and wide removal in one plane does not ensure complete excision. Fixation of cancer to adjacent structures mandates removal of the adherent area in continuity with the tumor. This is commonly seen with oral cancer that is firmly adherent to the underlying mandible or maxilla. Invasive cancer should not be peeled out, shelled out, enucleated, or curetted if a cure is expected. Many cancers are surrounded by a pseudocapsule. This capsule is almost invariably composed of compressed and viable tumor cells, *not* healthy reactive host cells. If a malignant tumor is entered at the time of resection, or if the margins of removal come back as incomplete from the pathologist, that procedure is often no better, therapeutically, than a large incisional biopsy. When possible, resection of the previous scar and the entire wound bed with "new" margins (never entering the old wound cavity) is indicated. One should strive for a level of dissection that is one tissue plane away from the mass (Figure 10-1). For example, invasion of cancer into the medullary cavity of a bone requires subtotal or total bone resection and not curettage.

4. Tumors should be handled gently to avoid risk of breaking off tumor cells into the operative wound, where they may thrive.[5] Copious lavage of all cancer wound beds will help mechanically remove small numbers of exfoliated tumor cells but should not replace gentle tissue handling and avoidance of entering the tumor bed.

5. If more than one malignant mass is being removed, separate surgical packs should be used for each site to avoid iatrogenic tumor cell implantation from site to site. Similarly, if the tumor bed was entered at the time of surgery, separate tumor packs should also be used for closure of normal tissues.

The aggressiveness of resection should only rarely be tempered by fears of wound closure. It is better to leave a wound partially open with no cancer than closed with residual cancer. Numerous innovative reconstructive techniques are available for closure of cancer wounds, and the surgeon is only limited by his or her ingenuity.[6] Reliable microvascular free composite transfers of muscle and skin are somewhat hampered due to unique canine skin/muscle anatomy but are being developed.[7]
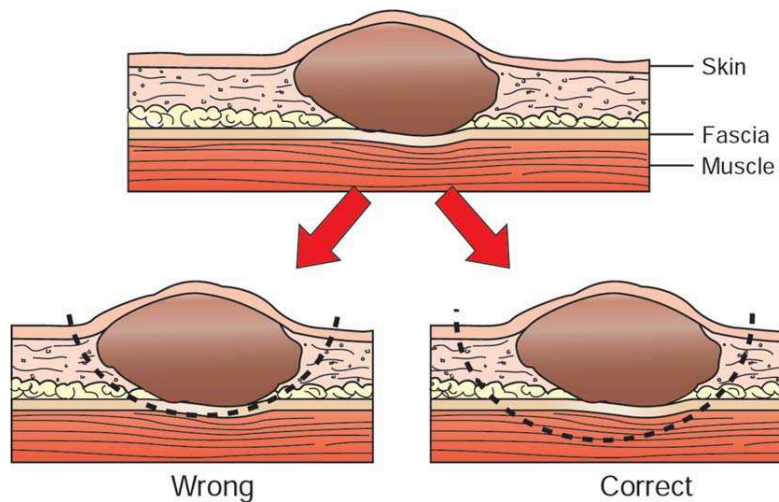
Figure 10-1

Typical soft tissue cancer is in proximity to skin and underlying fascia. Inappropriate removal is to "peel it off" the deeper fascia, where microscopic extension is probable. Correct removal entails wide margins three-dimensionally, including underlying skin and underlying fascia.

Metastasectomy for pulmonary metastasis of sarcomas has been accepted therapy in humans and dogs. Resection of liver metastasis for carcinomas (especially gastrointestinal cancers) is increasing in human oncology. As more effective adjuvant therapies evolve, the need for cytoreductive metastasectomy will increase.

LYMPH NODE REMOVAL

Controversy surrounds the surgical management of regional lymph nodes draining the primary tumor site.[8,9] As a general rule, epithelial cancers are more likely to metastasize to lymph nodes than are mesenchymal cancers. However, any enlarged regional lymph node requires investigation. Lymphadenopathy may be from metastasis of cancer (firm, irregular, and sometimes fixed to surrounding tissue) or from hyperplasia and reactivity to various tumor factors, infection, or inflammation.[10] The former cause is a poor prognostic sign, and the latter may be a beneficial host response. Enlarged lymph nodes as a result of cancer metastasis and invasion are generally uniformly effaced by tumor cells and can often be diagnosed by fine-needle aspiration. Histologically, positive lymph nodes usually are a sign of impending emergence of systemic metastasis. Lymph nodes should be removed under two general circumstances:

1. If the lymph node is positive for cancer and not fixed to surrounding normal tissues, it may be possible to remove the node with some therapeutic intent. Frequently, however, many lymph nodes

drain a primary tumor site (e.g., oral cavity) and lymphadenectomy is incomplete (e.g., neck dissection). Lymph node metastasis at the time of initial diagnosis is a poor prognostic sign. However, some patients develop metastasis in a delayed fashion (1 to 2 years) after local tumor control and may benefit from lymphadenectomy. Although it is usually not practical, removal of the primary tumor, intervening lymphatic ducts, and draining lymph node has been recommended (en bloc resection). En bloc resection may be possible for a malignant toe tumor with metastasis to the popliteal lymph node, but is usually only accomplished with amputation. A mastectomy that includes the regional lymph node is another example of en bloc resection. Few other anatomic sites are routinely amenable to this therapy. A specific instance where local lymphadenectomy may be beneficial is in removal of sublumbar lymph nodes in patients with metastatic apocrine or sebaceous gland adenocarcinomas of the perineum. Although removal of these lymph nodes is rarely curative, it may help increase the benefit of radiation therapy and should help alleviate, at least in the short term, the paraneoplastic syndrome of hypercalcemia by reducing levels of parathormone-like substances. It may also prevent or improve obstruction of the large bowel and urinary tract.

2. Normal-appearing lymph nodes that are known to drain a primary tumor site may be randomly sampled (biopsy or cytology) to gain further

staging information. This is particularly important if adjuvant therapy decisions (irradiation or chemotherapy) would be predicated on confirmation of residual or metastatic cancer. Intrathoracic or intra-abdominal lymph nodes are perhaps most crucial, since they are not readily accessible to histologic or cytologic follow-up examination. The emergence of sentinel node biopsy techniques in human medicine has allowed fewer "routine" nodal resections (especially the axillary nodes for women with breast cancer).[11] Basically, the area of the primary tumor is injected with blue dye or a low dose of a radionuclide or both. The first draining node is detected visually (dye) or with a handheld gamma camera probe and removed for a frozen section. If the first node is negative for metastasis, subsequent nodal dissection often is avoided. If the sentinel node is positive, further nodal dissection is performed. This technology could be applied to animal cancer patients.

Lymph nodes are *not* removed under two general circumstances:

1. Lymph nodes in critical areas (retropharyngeal, hilar, mesenteric) that have eroded through the capsule and become adherent (fixed) to surrounding tissues cannot be curatively removed without serious harm to the patient. They are best biopsied and left *in situ* or treated with other modalities. The occasional exception is metastasis of limb and paw tumors to prescapular and popliteal lymph nodes that can be removed with amputation (radical en bloc resection).
2. Prophylactic removal of "normal" draining lymph nodes or chains of lymph nodes (as opposed to sampling for stage) is of no benefit and may be harmful.[7] Regional lymph nodes may in fact be the initiator of favorable local and systemic immune responses, and elective removal has been associated with poor survival in certain human cancers.[8,12,13]

PALLIATIVE SURGERY

Palliative surgery is an attempt to improve the quality of the patient's life (pain relief or improved function) but not necessarily the length of the patient's life.[14] This type of surgery requires careful consideration of the expected morbidity of the procedure versus the expected gain to the patient and the client. In essence, it comes down to a decision of when to discontinue therapy. One of the most difficult decisions in surgical oncology is the decision *not* to operate. Treatment of any kind should never be worse than no treatment.

Certain situations do exist, however, where palliative surgery may be beneficial. If an infected and draining mammary tumor in a patient with asymptomatic lung metastasis is the limiting factor in the patient's life, mastectomy may still be a logical procedure. Splenectomy for ruptured hemangiosarcoma is commonly performed but probably has little impact on long-term survival and can be considered palliative, since it will stop the immediate threat of hemorrhage.

DEBULKING SURGERY

Incomplete removal of a tumor (planned or unplanned) is referred to as debulking or cytoreductive surgery. It is commonly performed but rarely indicated.[15] Its theoretical indication is to enhance the efficacy of other treatment modalities. Debulking is a practical consideration prior to cryosurgery to decrease the amount of tissue to freeze and the time it will take. It may also help the treatment planning and dosimetry with certain forms of irradiation, but the improved cancer control achieved is more a result of geometric and dosimetry considerations than removal of a few logs of tumor cells. Removing 99.9% of a 1-cm tumor (1×10^9 cells, one billion) still leaves a million cancer cells behind. Immunotherapy and chemotherapy could theoretically be helped by tumor volume reduction,[16,17] but few well-controlled clinical trials have shown a benefit to date in veterinary medicine. A variety of soft tissue sarcomas in dogs and cats will have better local control when radiation is given adjuvantly rather than for bulky measurable disease. Amputation or limb sparing of dogs with osteosarcoma is essentially a debulking procedure and clearly requires postoperative chemotherapy for prolongation of life. If tumors are debulked with the anticipation of postoperative radiation therapy, the margins of known tumor or the operative field should be marked with radiopaque metal clips to allow proper treatment planning from radiographs. The orientation of the incision should be considered carefully if radiation therapy is possible postoperatively.

SURGERY AND CHEMOTHERAPY

The combined use of chemotherapy and surgery is becoming more commonplace in veterinary oncology.[18,19] Many chemotherapy agents will impede wound healing to some extent. In spite of this risk, few clinically relevant problems occur when surgery is performed on a patient receiving chemotherapy.[20,21] General recommendations are to wait 7 to 10 days after surgery to begin chemotherapy, especially for high-risk procedures such as intestinal anastomosis.[22] The use of intraoperative[23] or perioperative chemotherapy is receiving increased attention[24,25] and could have greater implications for wound healing.

SURGERY AND RADIATION

Theoretical advantages can be advanced for both pre- and postoperative radiation.[19,26] Either way, some impairment of wound-healing potential will exist.[27] Radiation damage to normal tissues (stem cells, blood vessels, and lymphatics) is more permanent than chemotherapy damage. As total radiation dose, dose per fraction, and field size increase, the potential complications (with or without surgery) increase. If radiation therapy is given preoperatively, surgery can be performed after acute radiation reactions have resolved (generally 3 to 4 weeks). Postoperative radiation is recommended to start immediately or after a 10- to 14-day delay. In spite of the theoretical problems, surgery can be safely performed on irradiated tissues. Complications may occur but are not prohibitive.

PREVENTION OF CANCER

Certain common cancers in dogs and cats can be prevented. The recent elucidation of the canine genome as it relates to genetic susceptibility will likely increase the surgical indications for prevention. It is well known that early (<1 year) oophorectomy will reduce the risk of mammary cancer in the dog by 200-fold compared to intact bitches (and, to a lesser degree, the cat). Castration of the male dog will help prevent perianal adenomas, possibly prostatic adenocarcinoma, and obviously testicular cancer. Removal of *in situ* squamous cell carcinoma (precancerous) from the skin of white cats or removal of *in situ* adenomatous polyps from the rectum of dogs may also prevent subsequent development of cancer. Elective removal of cryptorchid testes, which are at high risk for tumor development, is another example of preventive surgery.

MISCELLANEOUS ONCOLOGIC SURGERY

Surgeons may be called upon to place long-term vascular access catheters for delivery of fluids, chemotherapy or anesthesia and pain relief agents. Operative placement of various enteral and parenteral feeding tubes is also commonly performed.

Surgeons and radiotherapists may work together for the operative exposure of nonresectable cancer so that large doses of irradiation may be delivered to the tumor or tumor bed after exclusion of radiosensitive tissues. Surgical intervention for oncologic emergencies such as intractable pain, bleeding, pathologic fracture, infection, and bowel perforation or obstruction may also arise.

Laparoscopic and thoracoscopic evaluation of body cavities for staging is increasingly being performed on animals. Cancer resections with this technique are also on the rise. The availability of equipment, further definition of appropriate case selection, and a steep learning curve will ultimately expand these techniques as the rising popularity of minimally invasive surgery is driving this technology.

DISCUSSION

It is clear that surgery will be the mainstay of cancer treatment in veterinary medicine for many years to come. It is also clear that just because a surgical procedure is possible, this is not the best reason to do it. It was not long ago that surgical resection of the external genitalia was routine treatment for dogs with transmissible venereal cell tumors. It is now recognized that chemotherapy alone is curative in over 90% of dogs and surgery is needed for biopsy only. Although rhinotomy and curettage of the canine nasal cavity can be performed, that may not improve survival over untreated patients.[28] However, one paper suggests that preoperative radiation followed by surgery in selected patients may be superior to radiation alone.[29] Likewise, simple versus radical mastectomy in the dog and humans does not influence survival for most mammary gland tumors, but more aggressive surgery may indeed be beneficial in the cat.[30–32] More surgery is not always better surgery. Long-term follow-up of well-staged and graded tumors with defined surgical technique and margins is necessary to demonstrate the true value of any operation. A great deal of progress in surgical technique and surgical thinking needs to take place before the use of surgery can be optimized. It is hoped that a better understanding of expected tumor biology and more precise staging methods (angiograms, ultrasound, CT scans, MRI, PET/CT, etc.) will facilitate more precise surgical operations to be performed. Surgical technique will continue to improve and undergo refinements,[33–36] but until surgeons become biologists, the big breakthroughs will be slow in coming. Surgeons should be investigating the influence of anesthesia, infection, immune function, blood transfusions, growth factors, oncogenes, and cytokines, to name a few, on the outcome of our patients.[37–43] In spite of these anticipated advances in technology and biology, the most difficult aspect to learn is surgical judgment. "Biology is King; selection of cases is Queen, and the technical details of surgical procedures are the Princes and Princesses of the realm who frequently try to overthrow the powerful forces of the King or Queen, usually to no long-term avail, although with some temporary apparent victories."[44]

The incorporation of the Veterinary Society of Surgical Oncology (VSSO) is further evidence of the maturity of surgical oncology in veterinary medicine. The VSSO should provide for well-defined surgical oncology trials and enhance teaching, training, research, and service in this critical area of oncology.

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Cancer Chemotherapy

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GENERAL PRINCIPLES OF CYTOTOXIC CHEMOTHERAPY

General Mechanism of Action

Both normal and neoplastic tissues contain cells that are actively proliferating and cells that are resting (see Chapter 2). The stages, or phases, of the cell cycle are the *S-phase*, the period of deoxyribonucleic acid (DNA) synthesis; the G_1 *phase*, during which ribonucleic acid (RNA) and protein synthesis occur; the *M-phase*, when mitosis occurs; and the G_2 *phase*, a second period of RNA and protein synthesis. Resting cells are in the G_0 phase. The mechanism of action of a chemotherapeutic drug can be cell cycle phase specific or nonspecific; that is, cytotoxic chemotherapeutic drugs can damage DNA, thereby preventing cellular replication or inducing apoptosis (or both), or they may interfere with a specific phase of the cell cycle. Because actively dividing cells are more sensitive to DNA damage and damage to ongoing cellular processes, chemotherapy is most effective against rapidly growing tumor masses.

Effects of the Biology of Tumor Growth

A major obstacle in the use of cancer chemotherapy is the fact that a tumor usually is far advanced before it is detected. For example, a tumor that is 1 cm in diameter contains about 10^9 cells. Another 10 doublings would produce 10^{12} cells, a mass that might measure 20 cm. A mass this size may be lethal, yet it could go unnoticed if located in the spleen or liver, for example. Unfortunately, a tumor this size likely would be poorly responsive to chemotherapy, because only about 5% of the tumor cells would be susceptible to treatment. The experimental evidence on which this observation is based suggests that the cells that are susceptible to most chemotherapeutic agents are the continuously dividing cells within the tumor. Cells in the resting phase (G_0 phase) and cells that are no longer able to divide but that contribute to the tumor volume commonly are "resistant" to chemotherapy. Cells in the G_0 phase can re-enter the cell cycle and begin dividing after a course of chemotherapy.

Tumor growth historically has been characterized by Gompertzian growth kinetics (Figure 11-1).[1] According to Gompertzian kinetics, the tumor growth fraction (the proportion of proliferating cells) is not constant. Tumor growth increases exponentially with time and then rapidly plateaus. Response to chemotherapy depends on where the tumor is in its growth curve. If the growth fraction is low, the fraction of cells killed by chemotherapy is small. Smaller tumors, which have a higher growth fraction, are more responsive to chemotherapy because they have a greater number of proliferating cells.

Indications for Chemotherapy

Chemotherapy is indicated in the following circumstances:

1. For patients with a measurable tumor known to be sensitive to chemotherapy (e.g., lymphoma, myeloma, or a transmissible venereal tumor [TVT])
2. As adjuvant therapy to eradicate occult micrometastasis (e.g., canine osteosarcoma or hemangiosarcoma)
3. As adjuvant therapy to prevent the recurrence of local tumor after incomplete surgical excision (e.g., vaccine-associated sarcoma in cats)
4. To downstage a chemosensitive tumor before definitive therapy (e.g., primary treatment or neoadjuvant treatment [chemotherapy delivered before the definitive modality] of an initially unresectable thymoma)
5. As palliative treatment to ease the clinical signs associated with unresectable or metastatic cancer (e.g., stranguria/pollakiuria associated with transitional cell carcinoma)
6. To sensitize tissues to radiation therapy

Even if the patient appears to have only localized disease, the biologic behavior of the tumor must be considered in the decision on whether to initiate chemotherapy. If the tumor is likely to metastasize,

Figure 11-1

Gompertzian growth kinetics. A hypothetical growth curve for a tumor showing the long latent period before detection and the progressive slowing of growth as the tumor enlarges. Tumor cells in the early latent growth period (high growth faction) tend to be more sensitive to chemotherapeutic agents. *(From Slingerland JM, Tannock IF: Cell proliferation and cell death. In Tannock IF, Hill RP, editors: The basic science of oncology, ed 3, New York, 1998, McGraw-Hill.)*

chemotherapy may be an appropriate part of the treatment plan.

The goal of most veterinary oncologists in using chemotherapy is to control the cancer and prolong survival while maintaining a good quality of life for the patient. It is important that the client understand the difference between a complete remission and a cure. For example, most dogs with lymphoma have a rapid, complete remission after initiation of chemotherapy; however, owners who do not understand that the lymphoma ultimately will relapse will be dissatisfied with their pet's treatment. The clinician is responsible for making sure that clients understand the goals of therapy for their particular pet before emotion, time, and money are invested in the treatment. Client education is extremely important, and a clearly defined treatment strategy should be outlined with the client. Handouts should be provided routinely to explain the rationale, toxicities, and handling of body fluids. Most dogs tolerate chemotherapy well, and in our experience, only a small percentage develop significant toxicity. Overall, studies assessing clients' perceptions of medical treatment for cancer in general and lymphoma in particular report a

positive experience; most owners felt that the treatment was worthwhile, that it resulted in improvement in their pet's well-being, and that the animal's quality of life during treatment was good.[2,3] Very few clients expressed regret about treating the lymphoma with a multidrug protocol.

Factors Affecting the Efficacy of Chemotherapy

The major factors that affect tumor cell kill by chemotherapy are as follows:
1. The duration of tumor exposure to an effective concentration of drug (i.e., drug concentration multiplied by length of exposure [$C \times T$]).
2. Specific and permanent biochemical resistance to the drug that is intrinsic (i.e., arises *de novo*) or acquired (i.e., develops after exposure to chemotherapeutic drugs)
3. Upregulation of drug-inactivating enzymes
4. Impaired transport of drugs across the cell membrane
5. Upregulation of cellular detoxification pathways, such as glutathione (GSH) and glutathione S-transferases (GSTs)
6. Dysregulation of apoptotic (cell death) or cell proliferation pathways (see Chapter 2)

An imbalance of the proapoptotic and antiapoptotic bcl-2–related proteins or activation of survival pathways by certain growth factors, such as insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF), may contribute to resistance. The tumor suppressor gene, p53, generally has been associated with enhanced apoptosis after genotoxic damage[4,5]; however, experimental evidence has raised some questions about the significance of p53 and chemotherapy sensitivity, particularly in solid tumors.[6]

A common form of acquired chemotherapy resistance arises through pleiotropic glycoprotein (P-glycoprotein), a transmembrane pump that removes xenobiotics from the cell cytoplasm. P-glycoprotein overexpression confers a simultaneous resistance to many different structurally unrelated drugs, including vinca alkaloids, epipodophyllotoxins, anthracyclines, dactinomycin, and the taxanes. P-glycoprotein is encoded by the multiple drug resistance (MDR) gene.[7] Although several drugs are good candidates for modulating MDR and are under investigation in human clinical trials, definitive results are lacking that would permit incorporation of these agents into standard protocols. Conversely, and of significant clinical importance, dogs with a defective MDR gene are more susceptible to the chemotherapeutic drugs affected by P-glycoprotein, and these patients show greater toxicity when given these agents (see Toxicity section later in the chapter).

An active debate has arisen among researchers over the mechanism by which drug-resistant clones develop. Figure 1-4 presents several possible models, which vary as to whether any tumor cell, or just the so-called tumor stem cell, has or acquires drug resistance.

Definitions of Commonly Used Terms

Clinicians should familiarize themselves with the terms commonly used in chemotherapy so that they are better able to understand procedures and treatments and explain them to clients.

Therapeutic index: The ratio of the dose of drug required to produce a given probability of toxicity and the dose required to give a defined effect against a tumor. The appropriate endpoints of tumor response and toxicity depend on the dose-limiting toxicity of the drug and the intent of treatment (i.e., cure or palliation).

Induction therapy: The induction phase of a chemotherapy protocol often involves more intensified therapy (shorter dosing intervals and more aggressive drug combinations) than the maintenance phase. The goal of induction therapy is to reduce the number of cancer cells and, ideally, induce a complete remission. For lymphoma, the role of long-term maintenance therapy has come under question (see Chapter 32).

Consolidation therapy (consolidation): Consolidation therapy is less intensive than induction therapy. It is designed to diminish further the number of cancer cells and to achieve complete remission in patients who did not go into complete remission following induction therapy.

Maintenance therapy: Maintenance therapy is a continuation of a less intensive chemotherapy protocol to maintain a remission and prevent relapse.

Salvage (rescue) therapy: Salvage therapy is reinduction chemotherapy for patients who fail one standard protocol. Drugs used in rescue protocols are not considered a first-line treatment for the particular tumor.

Complete remission (CR): Complete remission is complete resolution of measurable tumor based on physical examination, hematologic or biochemical monitoring, or diagnostic imaging. This designation is something of a moving target, because new molecular techniques have allowed investigators to determine "molecular remissions" rather than the previously standard clinical remissions.

Partial remission (PR): Under the new RECIST criteria,[8] the longest diameters of all target lesions are measured at 4 weeks after treatment and summed. A PR is defined as a reduction in the sum of the longest diameters of 30% or greater, with no new lesions developing, after initiation of chemotherapy.

Stable disease (SD, freedom from progression): Stable disease shows neither a PR nor progressive disease as measured at 4 weeks after initiation of chemotherapy.

Progressive disease (PD): Under the new RECIST criteria, progressive disease is defined as an increase in the sum of the longest diameters by 20% or greater as measured at 4 weeks or the development of new lesions.

Median duration of response/median survival: Median survival is the point at which half the patients have relapsed or died and half are still in remission and alive. This is not considered a practical measurement of treatment efficacy until results show a CR of 50% or greater.

Disease-free (relapse-free) interval (DFI) or *disease-free survival (DFS):* The disease- or relapse-free interval measures the time to progression of local or systemic disease after induction of a CR with surgery, radiotherapy, or chemotherapy. An example would be the time to metastasis after amputation for stage IIb osteosarcoma.

Adjuvant chemotherapy: Adjuvant chemotherapy is administered after surgery or radiotherapy. It is indicated in cases in which the risk of recurrence or metastases is high. Response to therapy can be difficult to measure, because often only microscopic disease exists; consequently, temporal measures of response (DFI, DFS) are used rather than size-based response criteria.

Primary (neoadjuvant) chemotherapy: Primary chemotherapy is used as the initial treatment (ideally for localized disease only) and then followed by some form of local therapy (surgery or radiotherapy). Measurable tumor mass has the least favorable cell kinetics (see Figure 11-1) and is most likely to contain resistant populations. Nevertheless, primary therapy may be useful when cytoreduction before local therapy is desired.

Maximal tolerated dose (MTD): The maximum recommended dose of a chemotherapeutic agent based on toxicity.

Nadir: The point in time after chemotherapy at which the lowest white blood cell (WBC) count occurs.

Combination Chemotherapy—the Best Chance for Tumor Control

The three most important elements for successful use of combination chemotherapy are (1) to administer the drugs in dosages as close to the MTD as possible; (2) to avoid overlapping drug toxicities; and (3) ideally, to use drugs that have known single agent activity against the tumor of interest. Although many tumors arise from a

single malignant cell, by the time they are clinically detectable (1 cm^3 or 10^9 cells), they contain a heterogeneous population of cells. By the time a tumor contains 10^6 cells, inherent drug resistance has developed. Goldie and Coldman[9] theorized that tumors develop intrinsically resistant cell lines, depending on their genetic instability. Clinically, this theory is observed as a tumor that initially responds to treatment with a PR or CR but then relapses as the resistant clone or clones grow in number. Researchers therefore have hypothesized that the induction regimen should be dose intensive and should involve multiple drugs, because single agent chemotherapy is not curative.[10] The tumor stem cell theory also could predict this clinical situation, because the committed cells are killed by the induction chemotherapy, but the tumor stem cells survive and repopulate the tumor.[11]

Combination protocols are designed according to the activity of the individual drugs against a specific tumor type, as well as drug toxicities and the drugs' mechanisms of action. Combination chemotherapy has three main objectives:

1. To maximize cell kill and maintain acceptable toxicities
2. To broaden the range of efficacy against a heterogeneous tumor population
3. To prevent or slow the development of resistant tumor cells

Comparison of published results of combination protocols, as well as the design of new combination chemotherapy protocols, can be challenging. *Summation dose intensity* is a concept that assumes linear efficacy and provides a numeric value to help researchers design new chemotherapy protocols and compare the dose intensities of previously reported studies.[12] Although this calculation requires a knowledge of maximally tolerated single agent doses, it assumes simple additive efficacy of drugs, and it does not take into account variations in tumor cell sensitivity to different chemotherapeutics; it is a basic method by which different chemotherapy protocols may be evaluated for dose intensity.

For example, a typical dose of cisplatin for the treatment of canine osteosarcoma is 70 mg/m^2 given intravenously (IV) once every 3 weeks. The "weekly" dose is 23.3 mg/m^2. Assuming that this is an MTD, the fractional dose intensity is 23.3 mg/m^2 divided by 23.3 mg/m^2, or 1. Doxorubicin is used to treat osteosarcoma at a dosage of 30 mg/m^2 given intravenously every 2 weeks; the "weekly" dose is 15 mg/m^2. A combination chemotherapy regimen for the treatment of canine osteosarcoma that uses cisplatin (50 mg/m^2 IV every 3 weeks) with doxorubicin (15 mg/m^2 IV every 3 weeks) has been reported.[13,14] The "weekly" doses for cisplatin and doxorubicin in this protocol are 16.7 mg/m^2 and 5 mg/m^2, respectively. The "weekly" fractional dose intensity of cisplatin is 16.7/23.3, or 0.72, and the "weekly" fractional dose intensity of doxorubicin is 5/15, or 0.33. Assuming linear efficacy, the summation dose intensity of the combination chemotherapy protocol is 1.05. This is essentially the same dose intensity as single agent cisplatin or doxorubicin; based on this number, the efficacy of the combination chemotherapy protocol is not likely to be greater than single agent therapy. To achieve a higher summation dose intensity, the protocol would require either higher drug doses or more frequent drug administration.

Toxicity

Most chemotherapeutic agents and protocols routinely used in veterinary oncology are well tolerated by most companion animals. These protocols generally are designed to result in less than a 5% hospitalization rate for chemotherapy toxicity and less than 1% mortality directly caused by toxicity. For the purpose of standardizing the reporting and analysis of chemotherapy-induced toxicities, the Veterinary Co-operative Oncology Group (VCOG) recently published a consensus document, "Common Terminology Criteria for Adverse Events" (VCOG-CTCAE; v1.0), for use after chemotherapy or biologic antineoplastic therapy in dogs and cats.[15] VCOG-CTCAE is a descriptive terminologic system that can be used to report an adverse event (AE); a grading (severity) scale is provided for each AE term.

General Side Effects

Gastrointestinal (GI) toxicity can occur secondary to direct damage to intestinal epithelial cells or by means of efferent nervous stimulation of the chemoreceptor trigger zone (CRTZ). It typically manifests as inappetence, nausea, vomiting, and/or diarrhea beginning 3 to 5 days after therapy. When direct stimulation of the CRTZ is responsible, vomiting is maximal on the day of therapy (Figure 11-2). The consequences of significant GI adverse events are many; they include dehydration, nutritional deficiency, delay of subsequent therapy, dose reduction, financial burden (i.e., hospitalization), and diminished client enthusiasm for continuation. Judicious use of antiemetics and antidiarrheals is recommended. If an animal has experienced significant GI events after a particular chemotherapy, the patient should be given 3 to 5 days of prophylactic therapy at subsequent treatment.

Myelosuppression occurs secondary to damage to the rapidly dividing bone marrow stem cells. Cells with the shortest circulating life span are most susceptible, therefore myelosuppression most often manifests as a decrease in the neutrophil or platelet count (or both). Specifically, chemotherapy treatment should be delayed

generally reveals marrow recovery. The use of human hematopoietic growth factors in animal species is controversial and rarely necessary in veterinary protocols. If the myelosuppressed animal is ill or pyretic, hospitalization and parenteral administration of broad-spectrum antibiotics, fluid support, and careful observation are indicated. A hunt for a source of infection, (e.g., occult pneumonia, urinary tract infection, local tumor necrosis or sepsis) may be indicated if clinical signs do not normalize rapidly with broad-spectrum antimicrobials and fluid support.

Typically, dose reductions of 20% are recommended for severe GI toxicity or if the neutrophil count falls below 500/μl at nadir or below 1500/μl at the time of the next scheduled treatment. Dose reductions should not be considered lightly, because dose intensity is extremely important for antitumor response. Symptomatic treatments (e.g., antiemetics, antidiarrheals) should be attempted to abrogate adverse effects of chemotherapy before dose reduction is considered.

Certain breeds, particularly collies, are known to be at risk for toxicity from chemotherapeutic drugs that are actively transported by the P-glycoprotein pump (e.g., vinca alkaloids, epipodophyllotoxins, anthracyclines, dactinomycin, and the taxanes).[16-19] These breeds have a high frequency of a pharmacogenetic mutation of the MDR1 allele (Table 11-1 lists the relative frequencies of the mutation in different geographic locations). If a dog is homozygous for the mutant allele, it is affected and at risk; if it is heterozygous for the mutant allele, it is a carrier. A clinically relevant polymerase chain reaction (PCR) assay (performed on cheek swabs) for the mutant status is available through Dr. Katrina Mealey's group at Washington State University Veterinary School. If a clinician is considering treating an at-risk breed, non-P-glycoprotein substrate chemotherapeutic drugs (e.g., alkylators) should be substituted in protocols until the results of the analysis are available (the turnaround time is approximately 1 week).

Alopecia is a common concern of owners, but it rarely is clinically significant. Dogs with continuously growing hair coats (e.g., poodles, terriers, and Old English sheepdogs) lose hair during chemotherapy (Figure 11-3). Clipped hair regrows slowly. Cats lose their guard hairs and whiskers (Figure 11-4) while undergoing chronic chemotherapy. The hair regrows once the patient has completed the protocol, but it may vary in color and texture.

Specific toxicities of individual drugs are addressed later in the chapter.

Safety of Chemotherapy

Studies assessing the exposure of human oncology nurses to cytotoxic drugs revealed increased amounts of

Figure 11-2

A dog showing classic signs of acute nausea after a chemotherapeutic infusion of cisplatin. Note the ropy saliva.

if the neutrophil count is 1500 to 2000/μl or less or if the platelet count is 50,000/μl or less. The exception to this rule is if the cytopenia is believed to have arisen secondary to the tumor, as a paraneoplastic syndrome or from myelophthisis. In either case, the neoplasia must be treated to resolve the low cell count.

Practitioners must keep in mind that most side effects of chemotherapy are self-limiting and resolve with minimal veterinary intervention. Most clients can perform the nursing care required to support animals through GI side effects. Moderate myelosuppression (1000 neutrophils/μl or less) in an otherwise clinically normal animal usually can be managed with prophylactic oral administration of antibiotics and body temperature monitoring. A repeat neutrophil count in 5 to 7 days

TABLE 11-1	Frequency of the MDR1 Pharmacogenetic Mutation in Breeds from Four Geographic Locations							
	UNITED STATES*		**FRANCE**[†]		**AUSTRALIA**[‡]		**Germany**[¶]	
Breed	H_T	H_O	H_T	H_O	H_T	H_O	H_T	H_O
Australian shepherd	29.8	1.7	—	—	42.8	21.4	25.2	6.9
Australian shepherd (mini)	44.6	3.6	—	—	42.8	21.4	—	—
Collie	46.8	31.2	32	48	64	24	43.1	33
Long-haired whippet	51.7	15.7	—	—	—	—	—	—
Sheltie	14.7	1.1	—	—	42.8	0	48.6	5.7

Neff MW, Robertson KR, Wong AK et al: Breed distribution and history of canine MDR1-1Δ, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage, Proc Natl Acad Sci U S A 101:11725-11730, 2004.

†*Hugnet C, Bentjen SA, Mealey KL: Frequency of the mutant MDR1 allele associated with multidrug sensitivity in a sample of collies from France, J Vet Pharmacol Therap 27:227-229, 2004.*

‡*Mealey KL, Munyard KA, Bentjen SA: Frequency of the mutant MDR1 allele associated with multidrug sensitivity in a sample of herding breed dogs living in Australia, Vet Parasitol 131:193-196, 2005.*

¶*Geyer J, Doring B, Goday JR, et al. Frequency of the nt 230 (del 4) MDR1 mutation in collies and related dogs breeds in Germany, J Vet Pharmacol Therap 28:545-551, 2005.*

H_T, *Heterozygous (carrier)*; H_O, *homozygous (affected)*.
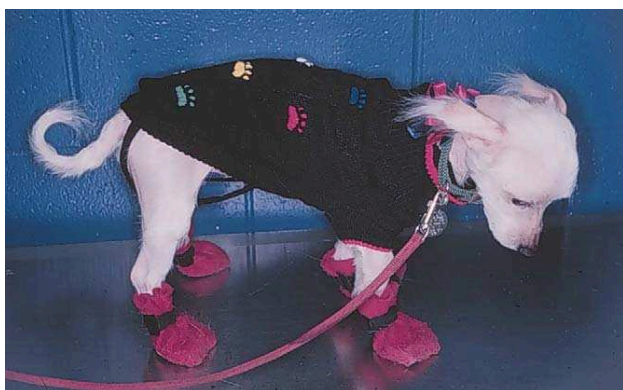
Figure 11-3

Near-complete alopecia in a Maltese undergoing chemotherapy for lymphoma.
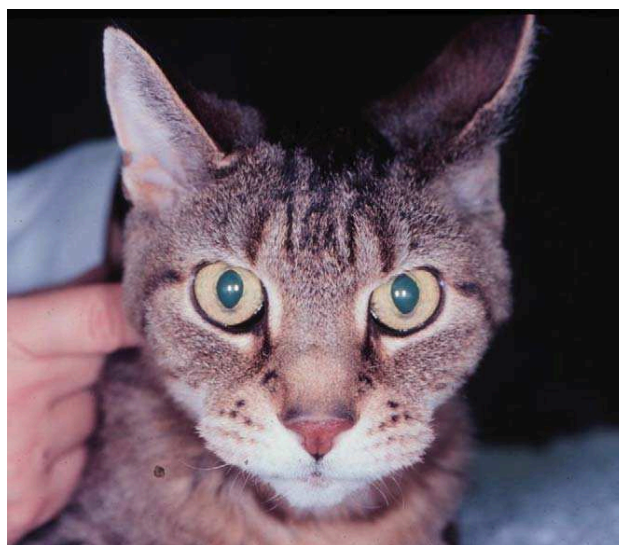
Figure 11-4

A cat undergoing chemotherapy for lymphoma who has lost his whiskers.

mutagens in urine concentrates and increased DNA damage in circulating lymphocytes.[20-23] Occupational exposure, therefore, has the potential to be hazardous. The severity and form of toxicity are related to the type, frequency, amount, and site of exposure, and several steps can be taken to minimize exposure (Table 11-2). Veterinarians do not require a special license to purchase and use chemotherapeutic drugs, but specific guidelines have been established for the storage and disposal of these medications. Safe handling of chemotherapeutic drugs is essential and relies heavily on common sense. The best way to avoid contamination is to follow proper storage and handling techniques. Chemotherapeutic drugs must be stored separately from all other medications and food items. A Class II biologic safety cabinet (vertical laminar airflow) should be used to reconstitute and draw up all injectable

Box 11-1	*Chemotherapy Safety Recommendations*

Safety Issue	**Recommendations**
To minimize the risk of topical contamination	1. Wear approved chemotherapy administration gloves. Latex examination gloves are not impermeable to chemotherapeutic agents. If chemotherapy administration gloves are not available, double-glove with latex exam gloves. 2. Wear a nonabsorbent chemotherapy administration gown or, at minimum, wear a buttoned-up lab coat. 3. Do not push air bubbles out of the syringe. 4. The use of commercially available chemotherapy dispensing systems (e.g., PhaSeal) can decrease the risk of exposure. 5. Use safety goggles or other protective eyewear.
To avoid the oral route of contamination	1. Never eat or drink in the chemotherapy administration room. 2. Never smoke or apply makeup in the chemotherapy administration room. 3. Never store chemotherapeutic drugs with food or other drugs. 4. Caution clients (and veterinary staff) always to wear gloves when administering chemotherapeutic drugs by the oral route.
Chemotherapy waste disposal	1. Separate chemotherapy waste from other sharps and biohazards, including needles, syringes, catheters, gloves, and masks. 2. Contact a local human hospital for aid in disposal of all chemotherapy-associated waste.
Precautions for patient care and clean-up (although the amount of active drug eliminated from the patient is minimal, it is prudent to take precautions)	1. Chemotherapeutic drugs are excreted in feces and urine: wear chemotherapy gloves when cleaning up after patients for 48 hours after drug administration. 2. No guidelines have been established for the disposal of pet waste; however, caution clients about cleaning up after their pets. If the patient urinates or defecates inside the home within 48 hours of receiving chemotherapy, owners should wear gloves to clean up the waste and should double-bag all waste.

chemotherapeutic agents. Chemotherapy safety gloves and gowns should be worn when these agents are handled. A new pharmacy-to-patient system, PhaSeal (Carmel Pharma, Columbus, OH), uses a double membrane setup to ensure leak-free transfer of drugs. Cytotoxic drugs have no contact with the atmosphere, and all connections remain dry. PhaSeal also uses an in-built pressure equalization technique. The expansion chamber ensures that neither overpressure nor a vacuum can occur during drug preparation; this effectively prevents vapor leakage. Other, similar systems likely will be marketed in the near future.

Veterinary clinics that do not have appropriate biosafety equipment should consider using a compounding pharmacy for individual prescriptions to minimize handling of the drugs beyond administration to the patient. See Box 11-1 for safety recommendations in the handling of chemotherapeutic drugs in the clinic and at home.[24]

SPECIAL USES OF CHEMOTHERAPY

Directed or Targeted Chemotherapy

The rationale for directed chemotherapy (intralesional, intracavitary, or inhalational form) is to attempt to achieve a higher concentration over time ($C \times T$) against the target tumor tissue while sparing normal tissue. In veterinary medicine, the most frequently used type of directed therapy is the intralesional form. Intralesional injection of a chemotherapeutic drug, usually administered in a poorly absorbed base such as pharmacologic-grade sesame oil, has been reported for squamous cell carcinomas in cats and horses and for oral melanomas and acanthomatous epulides in dogs. Cisplatin, carboplatin, bleomycin, 5-fluorouracil (5-FU), methotrexate, and BCNU all have been administered in this manner.[25-30]

Open-cell polylactic acid (OPLA)–platinum implants, used postoperatively in the tumor bed or

other subcutaneous sites, are another example of directed therapy that has been explored for use in dogs with osteosarcoma (see Chapter 23).[3,31,32] This system is a unique, slow-release method of administering chemotherapy that results in serum dose concentrations (area under the curve) that is more than 27 times that of native drug. Because the peak serum levels are much lower than with bolus dosing, fewer systemic toxicities develop than with native drug.

Intracavitary cisplatin (50 to 70 mg/m^2 every 4 weeks, combined with saline-induced diuresis) has been used effectively to reduce effusion with pleural mesothelioma and carcinomatosis.[33]

A novel method of administering chemotherapy is by inhalation using a jet nebulizer. In theory, use of this route should target the lungs and minimize systemic toxicity. In a study of 24 dogs with primary or metastatic lung cancer that was treated with inhalational doxorubicin or paclitaxel, five had a partial remission and one had a complete remission.[34] One dog with metastatic liposarcoma experienced long-term stabilization of the metastatic disease for longer than 12 months, and none of the dogs developed systemic toxicity.

Liposome Encapsulation

Liposomes are self-assembling colloidal particles in which a lipid bilayer encapsulates a fraction of the surrounding aqueous medium. Liposomes are available in several different forms, including conventional liposomes, polymorphic (cationic or fusogenic) liposomes, and nonreactive (sterically stabilized, pegylated, or "stealth") liposomes (SLs).

Conventional liposomes, which have a high delivery to monocytic and phagocytic cells, are used in veterinary oncology for the encapsulation of nonspecific immunostimulatory agents.[35-37] Polymorphic liposomes are used in gene therapy and immunotherapy (see Chapter 13).

Nonreactive liposomes are constructed of lipid bilayers that contain glycolipids or polyethylene glycol, which provide a steric barrier. When chemotherapeutic agents are encapsulated in such liposomes, the drug half-life (and therefore the length of tumor cell exposure to the drug) is greatly increased.[38] Stealth liposomes may also preferentially extravasate into sites where the vasculature is leaky (e.g., tumor beds).[39] SL-encapsulated doxorubicin (Doxil) has shown activity against mycosis fungoides, hemangiosarcoma, malignant histiocytoma, and lymphoma in dogs and against injection site sarcomas in cats.[40-42]

Liposomes also can be used to deliver drugs that are extremely toxic when administered in their free (nonencapsulated) form. For example, free cisplatin is lethal to cats; however, when it is encapsulated in a liposome, it can be administered safely.[43,44]

Chemotherapy and Radiation Therapy

The rationale for combining radiation therapy (see Chapter 12) and chemotherapy is to improve local control rates, reduce distant failures, and improve survival. In addition, animals that respond to this approach may have less of a need for radical surgery. The combination approach has been used to treat anal sac carcinoma with metastasis to regional lymph nodes, nasal tumors, bladder tumors, and oral and tonsillar squamous cell carcinomas. Chemotherapeutic agents such as cisplatin, mitoxantrone, gemcitabine, and paclitaxel have been used for radiosensitization, with mixed results.[45-49]

Drug Dosage and Timing

Traditionally, chemotherapeutic drugs have been given at the MTD and the shortest possible dosing interval (dose intensity). A dosage reduction as small as 20% can result in a decrease in drug efficacy of up to 50%.[50] Most chemotherapeutic agents are dosed on the basis of estimated body surface area (BSA) (Table 11-2). In dogs, the formula commonly used to estimate the BSA (m^2) from body weight is BSA $= 10 \times W^{2/3}$, where W is the body weight in grams. The validity of dosing chemotherapeutic agents based on BSA has been questioned with regard to increased toxicity in small dogs and cats (those that weigh less than 10 kg).[51] Increased toxicoses for low-body-weight animals have been observed with doxorubicin, cisplatin, carboplatin, and melphalan.[52-55] The current recommendation is to use body weight (mg/kg) to dose certain chemotherapeutic drugs (e.g., doxorubicin) in dogs that weigh less than 15 kg and in cats. Ideally, pharmacokinetic and pharmacodynamic information obtained for dogs of various body weights and for cats eventually will determine the optimal dosing administration for all chemotherapeutic agents.[53,56]

In metronomic chemotherapy, a more frequent (e.g., daily) low dose (well below the MTD) of the chemotherapeutic drug is given continuously, with no breaks in therapy.[57] With this delivery method, rather than administering the cytotoxic agent at the MTD, treatments are spaced so that normal tissues have sufficient time to recover. Significant *in vitro* and *in vivo* evidence (rodent tumor models) suggests that whereas conventional chemotherapy (using MTD dosing) targets tumor cells, metronomic chemotherapy may target growing tumor vascular endothelial cells and thus is antiangiogenic. Furthermore, because host vascular endothelial cells should be genetically stable and lack the diverse genetic defects characteristic of cancer cells that lead to drug resistance, the putative effects of long-term metronomic therapy might be more durable than those obtained with conventional dosing schemes.[57] The theorized

TABLE 11-2   Body Surface Area Conversion Charts (Body Weight in kg to m²)

WEIGHT TO BODY SURFACE AREA CONVERSION CHART—DOGS					
kg	m²	kg	m²	kg	m²
0.5	0.064	17.0	0.668	34.0	1.060
1.0	0.101	18.0	0.694	35.0	1.081
2.0	0.160	19.0	0.719	36.0	1.101
3.0	0.210	20.0	0.744	37.0	1.121
4.0	0.255	21.0	0.769	38.0	1.142
5.0	0.295	22.0	0.785	39.0	1.162
6.0	0.333	23.0	0.817	40.0	1.181
7.0	0.370	24.0	0.840	41.0	1.201
8.0	0.404	25.0	0.864	42.0	1.220
9.0	0.437	26.0	0.886	43.0	1.240
10.0	0.469	27.0	0.909	44.0	1.259
11.0	0.500	28.0	0.931	45.0	1.278
12.0	0.529	29.0	0.953	46.0	1.297
13.0	0.553	30.0	0.975	47.0	1.302
14.0	0.581	31.0	0.997	48.0	1.334
15.0	0.608	32.0	1.018	49.0	1.352
16.0	0.641	33.0	1.029	50.0	1.371

WEIGHT TO BODY SURFACE AREA CONVERSION CHART—CATS					
kg	m²	kg	m²	kg	m²
0.1	0.022	3.0	0.208	6.8	0.360
0.2	0.034	3.2	0.217	7.0	0.366
0.3	0.045	3.4	0.226	7.2	0.373
0.4	0.054	3.6	0.235	7.4	0.380
0.5	0.063	3.8	0.244	7.6	0.387
0.6	0.071	4.0	0.252	7.8	0.393
0.7	0.079	4.2	0.260	8.0	0.400
0.8	0.086	4.4	0.269	8.2	0.407
0.9	0.093	4.6	0.277	8.4	0.413
1.0	0.100	4.8	0.285	8.6	0.420
1.2	0.113	5.0	0.292	8.8	0.426
1.4	0.125	5.2	0.300	9.0	0.433
1.6	0.137	5.4	0.307	9.2	0.439
1.8	0.148	5.6	0.315	9.4	0.445
2.0	0.159	5.8	0.323	9.6	0.452
2.2	0.169	6.0	0.330	9.8	0.458
2.4	0.179	6.2	0.337	10.0	0.464
2.6	0.189	6.4	0.345		
2.8	0.199	6.6	0.352		

outcome of metronomic chemotherapy is stabilization rather than regression of disease. Also, chemotherapy administered in this fashion may have the advantage of being less toxic, because the drug doses used are well below the MTD. Combining metronomic chemotherapy with drugs known to have some inherent antiangiogenic activity (e.g., doxycycline) and cyclooxygenase-2 (COX-2) inhibitors (e.g., piroxicam) may increase the likelihood of an antitumor response. Although many veterinary oncologists are using metronomic protocols, no peer-reviewed articles on their efficacy or toxicity have been published; it must be stressed that the use of metronomic chemotherapy awaits validation through appropriate clinical trials.

SPECIFIC CHEMOTHERAPEUTIC AGENTS

Table 11-3 presents a summary of the commonly used chemotherapeutic drugs.

Alkylating Agents

Alkylating agents bind DNA strands, insert an alkyl group, and change the structure of DNA sufficiently to interfere with transcription, replication, and repair machinery. They therefore inhibit DNA, RNA, and protein synthesis. Some alkylating agents, such as melphalan, are bifunctional in that they contain two reactive sites. Alkylating agents are cell cycle phase nonspecific.

Carmustine. Carmustine (BCNU, BiCNU, Gliadel; Bristol-Myers Squibb Oncol, Guilford Pharmaceuticals; 100 mg powder for reconstitution or as a 7.7 mg wafer); compared to other chemotherapeutic drugs, carmustine is moderately expensive and moderately toxic.

Metabolism Carmustine has high lipid solubility and is essentially nonionized at physiologic pH. After IV administration, carmustine obtains excellent concentrations in the cerebrospinal fluid (CSF). Excretion is primarily through the urine, and a small percentage is released through the lungs as carbon dioxide (CO_2).

Dosage and Indications The dosage of carmustine for dogs is 50 mg/m² given IV over 15 to 20 minutes every 6 weeks. In veterinary medicine the primary indication for carmustine is treatment of inoperable brain tumors.[58] The dosage and indications for cats are unknown. Overall, little well-documented clinical information on this drug is available in the veterinary literature.

Toxicity In humans, the most common and serious side effects of carmustine are delayed myelosuppression and interstitial pneumonia.[58] Evaluation of a complete blood count (CBC) and platelet count every 7 days after drug administration is recommended; once a nadir has been established, monitoring need not be done this often. Nausea and vomiting may also occur. Specific toxicities of carmustine in cats are unknown.

Chlorambucil. Chlorambucil (Leukeran tablets; GlaxoSmithKline; 2 mg tablets); compared to other chemotherapeutic drugs, chlorambucil is moderately expensive and minimally toxic.

Metabolism After oral administration, chlorambucil is rapidly metabolized to its active form and is subsequently eliminated as inactive metabolites via the urine and feces.

Dosage and Indications The dose for chlorambucil varies, depending on the protocol. The drug is used in

TABLE 11-3 Commonly Used Chemotherapeutic Drugs

Drug	Main Indications	Toxicities	Dosage
Alkylating agents			
Cyclophosphamide	Lymphoma, carcinoma, sarcoma	Marrow, gastrointestinal (GI) tract, sterile hemorrhagic cystitis	Given orally (PO) or intravenously (IV); many dosing regimens can be used, depending on concurrent anticancer drugs.
Chlorambucil	Lymphoma, chronic lymphocytic leukemia, mast cell tumor, IgM myeloma Substitute for cyclophosphamide if hemorrhagic cystitis occurs.	Mild marrow toxicity	Given PO only; many dosing regimens can be used, depending on concurrent anticancer drugs.
CCNU (lomustine)	Relapsed lymphoma or mast cell tumor; brain tumor	Myelosuppression and idiosyncratic, potentially fatal hepatotoxicity	Dogs: 60-90 mg/m² PO every 3 weeks Cats: 50-60 mg/m² PO every 3-6 weeks

TABLE 11-3   Commonly Used Chemotherapeutic Drugs—cont'd

Drug	Main Indications	Toxicities	Dosage
Alkylating agents—cont'd			
Dacarbazine	Lymphoma	Myelosuppression, vomiting during administration, perivascular irritation upon extravasation	Dogs: 200 mg/m^2 IV daily for 5 days every 3 weeks *or* 1000 mg/m^2 IV every 3 weeks
Ifosfamide	Lymphoma	Hemorrhagic cystitis, myelosuppression	Dogs: 275-350 mg/m^2 IV with saline diuresis and mesna, every 3 weeks
Melphalan	Multiple myeloma, anal sac adenocarcinoma	Myelosuppression, potential cumulative thrombocytopenia	Dogs: 0.1 mg/kg every 24 hours for 10 days, then 0.05 mg/kg daily *or* 7 mg/m^2 PO daily for 5 days every 3 weeks Cats: 0.1 mg/kg every 24 hours
Anthracyclines			
Dactinomycin	Lymphoma	Myelosuppression, GI upset, perivascular damage with extravasation	0.75-0.8 mg/m^2 IV every 3 weeks
Doxorubicin	Lymphoma, carcinoma, sarcoma	Myelosuppression, GI upset, hypersensitivity during administration, perivascular damage with extravasation, cumulative (180 mg/m^2) myocardial toxicity, nephrotoxicity (cats)	Dogs ≥10 kg: 30 mg/m^2 IV every 2-3 weeks Dogs <10 kg: 1 mg/kg IV every 2-3 weeks Cats: 1 mg/kg IV every 3 weeks
Doxorubicin HCl liposome injection	Lymphoma, carcinoma, sarcoma	Mild myelosuppression and/or GI upset, hypersensitivity during administration, perivascular damage with extravasation, palmar plantar erythrodysesthesia (PPES) nephrotoxicity (cats)	Dogs and cats: 1 mg/kg IV every 3 weeks
Idarubicin	Unclear	Mild myelosuppression and/or GI upset, perivascular damage with extravasation	Idarubicin: 2 mg/cat/day for 3 days q 3 wk
Mitoxantrone	Lymphoma, transitional cell carcinoma	Myelosuppression, GI upset, perivascular damage with extravasation	Dogs: 5-5.5 mg/m^2 IV every 3 weeks Cats: 6 mg/m^2 IV every 3 weeks
Antimetabolites			
Methotrexate	Lymphoma	Mild myelosuppression and/or GI upset	Given PO or IV Dogs and cats: 0.8 mg/kg in combination with other chemotherapeutic drugs
Cytosine arabinoside	Lymphoma (myeloproliferative)	Mild myelosuppression and/or GI upset	Given subcutaneously (SQ), intramuscularly (IM) or IV; several different regimens can be used, depending on concurrent anticancer drugs.

Continued

TABLE 11-3   Commonly Used Chemotherapeutic Drugs—cont'd

Drug	Main Indications	Toxicities	Dosage
Antitubulin agents			
Paclitaxel	Under investigation	Hypersensitivity during administration	Dogs: 132 mg/m^2 IV every 3 weeks; must premedicate to minimize hypersensitivity
Vinblastine	Mast cell tumor	Myelosuppression, perivascular vesicant	Dogs: 2 mg/m^2 IV every 1-2 weeks
Vincristine	Lymphoma, mast cell tumor, transmissible venereal tumor, immune-mediated thrombocytopenia	Myelosuppression, perivascular vesicant, peripheral neuropathy, constipation in cats	Dogs and cats: 0.5-0.7 mg/m^2 IV weekly or as dictated by concurrent anticancer drugs.
Vinorelbine	Primary lung tumor	Myelosuppression, perivascular vesicant	Dogs: 15-18 mg/m^2 IV every 1-2 weeks
Corticosteroids			
Prednisone	Lymphoma, mast cell tumor, myeloma, chronic lymphocytic leukemia, Noncytotoxic indications: brain tumor, insulinoma, appetite stimulant	Polyuria, polyphagia, polydipsia, muscle wasting, behavioral changes	Dogs and cats cytotoxic dose: 2 mg/kg/day, taper according to protocol Dogs and cats noncytotoxic dose: 0.5 mg/kg/day
Miscellaneous drugs			
Asparaginase	Lymphoma	Hypersensitivity reaction after administration	Dogs and cats: 400 IU/kg SQ or IM, maximum dose of 10,000 IU
Carboplatin	Osteosarcoma, carcinoma, sarcoma	Myelosuppression; potentially severe (small dogs) GI effects	Dogs: 300 mg/m^2 IV every 3 weeks Cats: 240-260 mg/m^2 IV every 3 weeks
Cisplatin	Osteosarcoma, carcinoma, sarcoma	Nephrotoxic—must be given with saline-induced diuresis; highly emetogenic; fatal to cats	Dogs: 70 mg/m^2 IV every 3 weeks Cats: Do not use.
Hydroxyurea	Polycythemia vera, myeloproliferative diseases	Myelosuppression	Dogs: 50 mg/kg/day, tapering to every other day with remission Cats: 10 mg/kg/day, tapering to every other day with remission
Procarbazine	Lymphoma	GI upset, myelosuppression	Dogs: 50 mg/m^2 daily for 14 days on and 14 days off as part of mechlorethamine, Oncovin (vincristine), procarbazine, and prednisone (MOPP) protocol

the maintenance phase of some lymphoma protocols.[59,60] It also may be used as a substitute for cyclophosphamide in a lymphoma protocol if sterile hemorrhagic cystitis occurs. When it is substituted for cyclophosphamide in the cyclophosphamide, Oncovin, and prednisone (COP) protocol, the dosage for both dogs and cats is 0.8 mg/kg given orally once on the same schedule as cyclophosphamide.[61] When the drug is used in the University of Wisconsin–Madison lymphoma protocol, the dosage is 1.4 mg/kg given orally all on the same day.[59] Chlorambucil is also used for small cell variants of lymphoma in dogs and cats and for immunoglobulin M (IgM)-secreting myeloma in dogs (see Chapter 31, sections A, B, and D).

Toxicity Potential toxicities of chlorambucil include myelosuppression, nausea, and vomiting.

Cyclophosphamide. Cyclophosphamide (Cytoxan for injection, Cytoxan tablets, Neosar; Gensia Sicor, Mead Johnson Oncology; 25 and 50 mg tablets and 100, 200, 500 mg and 1 and 2 g powder for reconstitution); compared to other drugs, cyclophosphamide is relatively inexpensive and moderately toxic.

Metabolism Cyclophosphamide is a prodrug that is activated in the liver by mixed-function oxidase enzymes into 4-hydroxycyclophosphamide and which aldophosphamide; both are active forms of the drug. Byproducts of cyclophosphamide metabolism are carboxyphosphamide, acrolein, and phosphoramide mustard.[62] Excretion of all metabolites occurs primarily through the urinary system.

Dosage and Indications Cyclophosphamide is supplied as a sterile powder for injection and in tablet form for oral administration. The tablets should not be split, because the active ingredient is not dispersed evenly throughout the tablet, and the chemotherapy safety hazard is greater with exposed drug at the cut surface. Some pharmacists will compound the drug into smaller dose capsules. Administration protocols for dogs and cats vary, ranging from a single large bolus dose to division of the dose over 4 days; the total dose ranges from 200 to 300 mg/m^2. If the drug is given over 4 days, the total dose for that period must be calculated accurately; if it cannot be divided evenly over 4 days, it can be adjusted to be given over 3 days. For example, if a patient's weekly dose is 150 mg (37.5 mg for 4 days), the practitioner or client should *not* try to administer one and one half 25 mg tablets daily; rather, one 50 mg tablet should be given once daily for 3 days, resulting in a total dose of 150 mg. Cyclophosphamide is combined with other chemotherapeutic drugs to treat lymphoma and various carcinomas and sarcomas.[37,63-67]

Toxicity The primary side effect of cyclophosphamide is bone marrow suppression. Another important toxicity is bladder irritation caused by the inactive metabolite acrolein, resulting in sterile hemorrhagic cystitis (SHC).[68-70] This problem can arise after a single dose or after long-term administration of the drug.[68,70] Dogs that have received cyclophosphamide should be encouraged to drink fresh water and should be let outside to urinate several times a day for 2 days after each treatment. Diuresis, either through administration of subcutaneous or IV fluids or therapy with corticosteroids or furosemide, is another preventive measure.[70] Concomitant administration of sodium-2-mercaptoethanesulfonate (mesna; Mesnex, Bristol-Myers Squibb), a uroprotective agent, is used in humans treated with cyclophosphamide or ifosfamide, a newer alkylating agent.[70] Because of the relatively low incidence of SHC associated with cyclophosphamide in dogs, mesna is not routinely administered to veterinary patients. An important differential diagnosis for SHC is bacterial cystitis; a full urinalysis and culture should always be performed in a patient with clinical signs of cystitis. SHC typically is self-limiting and usually resolves within weeks to months after cessation of cyclophosphamide therapy.[69,70] Although no specific treatment exists, oxybutynin chloride (0.2 to 0.3 mg/kg given orally 2 to 3 times daily) may help relieve the clinical signs. Once cystitis develops, cyclophosphamide should not be administered again; in patients with lymphoma, chlorambucil should be used as a substitute.[65]

Dacarbazine. Dacarbazine (DTIC-Dome; Bayer, various pharmaceutical companies; 100 and 200 mg powder for reconstitution); compared to other drugs, dacarbazine is moderately expensive and moderately well tolerated.

Mechanism of Action The exact mechanism of action of dacarbazine is unknown; it most likely acts as an alkylating or cross-linking agent.

Metabolism Dacarbazine is metabolized and eliminated through the biliary and renal systems.

Dosage and Indications Dacarbazine can be dosed in dogs at 200 mg/m^2 as a slow IV bolus given once daily for 5 days, or once as a slow IV infusion (over 2 to 8 hours) at 1000 mg/m^2 every 3 weeks.[71-73] The drug should be administered through a perfectly placed IV catheter, because dacarbazine is a vesicant. In veterinary patients, the primary indication for dacarbazine is in combination with doxorubicin or dactinomycin for the treatment of relapsed lymphoma; however, one report exists of a complete remission in a dog with cutaneous T-cell lymphoma treated solely with dacarbazine.[71-74] The dosage and indications for cats are unknown.

Toxicity Side effects of dacarbazine include nausea, vomiting, anorexia, myelosuppression, and severe

tissue necrosis if accidental extravasation occurs. Anecdotally, more than 50% of dogs treated with an 8-hour infusion protocol vomit during administration, and 30% of dogs show agitation. Prophylactic antiemetics are recommended for dogs dosed at 800 to 1000 mg/m^2.[73] Hematologic parameters (CBC, platelet count) should be monitored before each dacarbazine administration and again 7 to 10 days later. In one study, the combination of doxorubicin with dacarbazine caused sepsis in 15% of the animals treated.[73]

Ifosfamide. Ifosfamide (Ifex; Mead Johnson Oncology; 1 and 3 g single-dose powder for reconstitution, packaged with mesna, the mandatory uroprotective agent); compared to other drugs, ifosfamide is expensive and, when administered with saline-induced diuresis and mesna, moderately toxic.

Metabolism Ifosfamide, which is an isomer of cyclophosphamide, is excreted through the urinary tract. It must be given in conjunction with 2-mercaptoethane sulfonate (mesna) to prevent hemorrhagic cystitis.

Dosage and Indications The recommended dosage of ifosfamide is 350 to 375 mg/m^2 given intravenously every 3 weeks.[75] In one study, 72 dogs with a variety of tumors were evaluated for response to ifosfamide; a disappointing overall response rate of 6% was reported, and most positive responses occurred in dogs with sarcomas, including cutaneous hemangiosarcoma and leiomyosarcoma.[75] A protocol alternating ifosfamide with doxorubicin for the treatment of 27 dogs with hemangiosarcoma failed to show any improvement in survival compared to surgery alone.[76] Only one of 40 dogs with lymphoma responded favorably to ifosfamide.[75] The dosage and indications for cats are unknown. The role of ifosfamide in veterinary oncology remains to be determined.

Toxicity Ifosfamide is less myelosuppressive than cyclophosphamide but much more urothelial toxic; mesna is always recommended in conjunction with this drug. Mesna is a thiol compound that protects against SHC by binding and thus inactivating acrolein, a urotoxic metabolite. Mesna is administered intravenously at 20% of the dose of ifosfamide; it is given at the time of ifosfamide administration and also 2 and 5 hours later.[63] If mesna is used to control cystitis, the dose-limiting toxicity is myelosuppression.[75]

Lomustine. Lomustine (CCNU, CeeNU; Bristol-Myers Squibb; 10, 40, and 100 mg capsules); compared to other drugs, lomustine is moderately expensive and moderately toxic.

Metabolism Lomustine is metabolized in the liver by the hepatic microsomal enzyme oxidation system and excreted in the urine.

Dosage and Indications The dosage of lomustine for dogs is 60 to 90 mg/m^2 given orally once every 3 weeks. Lomustine has been used to treat brain tumors.[77,78] It also is used as a rescue agent for resistant lymphoma and has been used in the treatment of canine mast cell disease.[79-82] In one study in cats, because lomustine is available in 10 mg capsules, a dosage of 10 mg/cat (range, 32 to 59 mg/m^2) was given every 3 weeks and was well tolerated, the primary toxicity being myelosuppression.[80] Another study in cats reported safe use of a dosage range of 50 to 60 mg/m^2, with prolonged neutropenia the most frequent toxicity.[83] Some cats could be dosed only every 6 weeks because of myelosuppression. Both papers reported antitumor effects against lymphoma, and occasional responses against fibrosarcoma, mast cell tumors, and multiple myeloma also were noted.

Toxicity Myelosuppression in dogs is seen at 7 to 14 days and may be severe. Dogs and cats may experience delayed and cumulative neutropenia and thrombocytopenia, and dogs may also develop potentially fatal hepatotoxicity.[79-81,84] A retrospective study of 179 dogs treated with lomustine reported that 11 dogs (6.1%) experienced hepatic toxicity, and seven of these dogs died of progressive liver failure.[84] Greater numbers of treatments were more likely to be associated with hepatopathy. Liver enzymes, particularly alanine aminotransferase (ALT), should be monitored throughout lomustine therapy, and the drug should be discontinued if ALT elevation occurs.

Mechlorethamine. Mechlorethamine (Mustargen; Merck; 10 mg powder for reconstitution); compared to other drugs, mechlorethamine is moderately expensive and, as part of the mechlorethamine, Oncovin (vincristine), procarbazine, and prednisone (MOPP) protocol, moderately well tolerated.

Mechlorethamine is one of the original chemotherapeutic drugs. It is derived from mustard gas, which was used as a weapon in World War I. Victims of mustard gas showed severe depletion of white blood cell populations and atrophy of the bone marrow and lymphatic tissues. One of the first derivatives of mustard gas was the nitrogen mustard mechlorethamine. In the 1940s, mechlorethamine was shown to have positive effects in patients with lymphoma, resulting in the introduction of chemotherapy into clinical practice.

Metabolism Mechlorethamine undergoes rapid hydrolysis to active metabolites in plasma and is eliminated through the urinary tract.

Dosage and Indications The dosage for mechlorethamine in dogs is 3 mg/m^2, given intravenously as a rapid bolus.[85] The drug is supplied as a lyophilized powder. Once reconstituted, it should be

administered within 20 minutes, and unused drug should be discarded. A major concern in the use of this drug is its intermittent limited availability. In veterinary medicine, mechlorethamine is used as part of the MOPP protocol for the treatment of lymphoma in dogs. In one study, when this protocol was used to treat dogs with relapsed lymphoma, the overall response rate was 65%; however, as with most rescue protocols, the responses were not durable.[85] The dosage and indications for cats are unknown.

Toxicity Mechlorethamine is irritating if injected perivascularly, therefore a well-placed catheter must always be used to administer the drug. The toxicities of single agent mechlorethamine are unknown. The most common toxicity of the MOPP protocol is GI effects; as many as 13% of patients require hospitalization.[85] Neutropenia is another potential toxicity of the protocol. In one study, five of 117 dogs developed sepsis, and two of the 117 died as a result of neutropenia and sepsis.[85]

Melphalan. Melphalan (Alkeran tablets, melphalan for injection; Celgene; 2 mg tablets or 50 mg vial for injection); compared to other drugs, melphalan is relatively inexpensive and well tolerated.

Metabolism Melphalan undergoes rapid hydrolysis in plasma to active metabolites, which are eliminated in the urine and feces.

Dosage and Indications Melphalan most often is used with prednisone to treat multiple myeloma.[86,87] A dosing recommendation that may be used in dogs or cats is 0.1 mg/kg daily for 10 days, then 0.05 mg/kg every other day indefinitely.[88] Pulse dose administration of melphalan (7 mg/m² given orally daily for 5 days, every 3 weeks) is successful in some cases that do not respond to daily doses. At least anecdotally, the pulse dose protocol is less likely to produce cumulative delayed thrombocytopenia.

Toxicity Although melphalan produces few side effects, myelosuppression may occur. Thrombocytopenia may be seen with chronic use. The CBC should be checked every week initially and then every 4 to 8 weeks. When pulse dosing is used, a CBC must be done before the start of each 5-day treatment.

Streptozocin. Streptozocin (Zanosar; Pharmacia; 1 g powder for reconstitution); compared to other drugs, streptozocin is expensive and moderately toxic.

Metabolism Streptozocin is converted in the liver into active metabolites, which are eliminated through the urinary tract.

Dosage and Indications Streptozocin is directly toxic to pancreatic beta cells and therefore is potentially useful in the management of insulinoma.[89] The dosage in dogs is 500 mg/m² given intravenously every 3 weeks, administered with a 0.9% saline–induced diuresis of 18.3 ml/kg/hr for 3 hours before administration of the chemotherapy. The drug, diluted in saline to continue the 18.3 ml/kg/hr rate, is given over the next 2 hours, and the patient then continues on the saline-induced diuresis for another 2 hours after completing the chemotherapy infusion.[89] The dosage and indications for cats are unknown.

Toxicity The major toxicity of streptozocin is renal tubular necrosis; administration with the previously described saline-induced diuresis is essential to protect the kidneys.[89] The renal tubular necrosis is dose related and cumulative. The drug is emetogenic; butorphanol (0.4 mg/kg given intramuscularly) may help minimize vomiting. Elevation of liver enzymes may also occur with streptozocin administration, but myelosuppression is rare. Because the drug is toxic to insulin-secreting cells, diabetes mellitus may be a sequela to therapy.[89]

Antibiotics

Dactinomycin. Dactinomycin (Actinomycin-D, Cosmegen; Merck; 0.5 mg lyophilized powder for reconstitution); compared to other drugs, dactinomycin is inexpensive and well tolerated.

Mechanism of Action Dactinomycin is an antitumor antibiotic originally identified as a product of the *Streptomyces* yeast species. Its mechanism of action is through DNA intercalation and inhibition of RNA and protein synthesis, and it is cell cycle phase nonspecific. After IV administration, the drug concentrates in nucleated cells; it does not penetrate the blood-brain barrier, and it is minimally metabolized. Excretion occurs through urine and feces.

Dosage and Indications The recommended dosage of dactinomycin in dogs is 0.5 to 0.9 mg/m² given intravenously once every 2 to 3 weeks.[71,90,91] The calculated dose can be given as an IV bolus or diluted in up to 20 ml of 0.9% sodium chloride or D₅W and given over 5 to 10 minutes through an indwelling IV catheter. Dactinomycin is used as a rescue agent for patients who have failed more conventional chemotherapy protocols for lymphoma, although its efficacy is questionable.[90-92] It also is used as a noncardiotoxic substitute for doxorubicin in multidrug lymphoma protocols when a cumulative dose of 180 to 240 mg/m² of doxorubicin has been reached, although it is less efficacious than doxorubicin.[71,92] In one study, partial responses to dactinomycin were seen with various carcinomas, including anal sac and perianal adenocarcinomas, squamous and transitional cell carcinomas, and a thyroid

carcinoma.[91] Dactinomycin has also been used in dogs with nephroblastomas. The dosage and indications for cats are unknown.

Toxicity Dactinomycin is a vesicant and must be administered through a carefully placed IV catheter. GI toxicity is the most common complication and may manifest as nausea, vomiting, and/or diarrhea. Moderate myelosuppression may occur; a CBC and platelet count should be evaluated 7 to 10 days after administration of the drug.[91,92] Significantly increased neurotoxicity of 5-FU has been reported when it is co-administered with dactinomycin and cyclophosphamide.[90]

Doxorubicin. Doxorubicin (Adriamycin PFS or RDF; Pharmacia & Upjohn, Bedford Labs; 10, 20, and 50 mg single-dose vials); compared to other drugs, doxorubicin is inexpensive and moderately toxic.

Mechanism of Action Doxorubicin is an anthracycline derived from the *Streptomyces* yeast species. It has multiple mechanisms of action, including intercalation of DNA, leading to inhibition of protein synthesis and free radical formation, and inhibition of topoisomerase enzymes. It is cell cycle phase nonspecific. Doxorubicin is extensively metabolized by the liver and is eliminated primarily in the feces.

Dosage and Indications In dogs, doxorubicin normally is administered intravenously at a dosage of 30 mg/m² given once every 2 to 3 weeks. Because of concerns about overdosing of small dogs on a m² basis, a dosage of 1 mg/kg can be used in dogs that weigh 10 kg or less.[51] In cats, a dosage of 20 to 25 mg/m² or 1 mg/kg is used.[93,94] Doxorubicin can cause hypersensitivity reactions; slow administration (0.5 ml per minute) may abrogate this reaction. A slower IV infusion also has been reported to provide a greater area under the time-versus-concentration curve; however, the clinical significance of this is unkown.[95] Because the drug causes severe perivascular necrosis and tissue sloughing with extravasation (Figure 11-5), use of a perfectly placed, "first stick" IV catheter for administration is mandatory. Dogs undergoing doxorubicin infusions should not be left alone during the infusion; constant observation until completion is necessary to ensure a patent catheter and successful infusion. Doxorubicin has a wide range of antitumor activity. Common indications include lymphoma, osteosarcoma, hemangiosarcoma, and a variety of carcinomas.[65,66,94,96-100]

Toxicity An understanding of the side effects of doxorubicin is crucial. Acute effects include a hypersensitivity reaction, extravasation injury or, in rare cases, arrhythmias.[101,102] Chronic, cumulative effects include cardiomyopathy (primarily in dogs) and nephrotoxicity

in cats.[102,103] Compared to other chemotherapeutic drugs, doxorubicin is more likely to cause GI upset, myelosuppression, and alopecia. Animals undergoing doxorubicin chemotherapy should be monitored accordingly.

Dogs and cats may develop immediate, histamine-mediated side effects that manifest as allergic reactions and possibly shock. Allergic reactions in the dog are manifested in the GI tract and the skin; these two organs are most severely affected during and immediately after administration of doxorubicin. In the cat, the lungs are more likely to be affected. If adverse acute drug reactions occur (e.g., pruritus, wheals, vomiting, restlessness, or dyspnea), the practitioner should stop the doxorubicin infusion, administer diphenhydramine (3 to 4 mg/kg given intramuscularly) and dexamethasone SP (0.5 to 1 mg/kg given intravenously), and wait for the reaction to subside before restarting the doxorubicin infusion at a slower rate. Animals that demonstrate severe acute signs should be premedicated with diphenhydramine and dexamethasone 15 to 20 minutes before subsequent doxorubicin treatments.

Doxorubicin is a potent vesicant; it is extremely damaging if extravasated. Surgical treatment, including immediate debridement of the extravasated tissues and possibly amputation, may be required. Dexrazoxane has been shown to abrogate the tissue damage associated with extravasation if given immediately after

Figure 11-5

A dog 2 weeks after doxorubicin extravasation. Severe perivascular and soft tissue necrosis has occurred, and the dog eventually had to undergo an amputation because of the extravasation.

extravasation (and possibly within 3 hours of infusion).[101] Anecdotally, IV administration of dexrazoxane at 10 times the dose of doxorubicin within 3 hours and again at 24 and 48 hours after extravasation significantly reduces local tissue injury.

Arrhythmias are a rare acute manifestation of cardiac toxicity.[102] In the authors' experience, clinically evident doxorubicin-associated arrhythmias are transient. A cardiac issue of greater concern is doxorubicin-induced myocardial toxicity arising from the generation of iron-dependent oxygen free radicals; the levels of enzymes that detoxify these free radicals are lower in the myocardium.[102] No good screening test is available to predict which dogs will develop cardiotoxicity, although breeds predisposed to dilated cardiomyopathy should have a baseline fractional shortening measurement before starting doxorubicin. Sequential echocardiography and electrocardiography may be used to monitor for changes in fractional shortening, but they are very poor predicators of whether or when an animal will develop heart failure.[104] Endomyocardial biopsy is the monitoring test of choice for doxorubicin-associated cardiomyopathy, but it is not routinely performed, for obvious reasons. The current recommendation, that the cumulative dose should not exceed 180 to 240 mg/m², is a guideline that should be applied to each patient individually.[105] The client should be informed of the significant risks associated with high cumulative doses of the drug, but a careful balance must be struck between the potential therapeutic benefit and toxicity. When doxorubicin is the only effective agent or if an animal quickly relapses without the drug, exceeding the "recommended" drug levels may be appropriate to prevent death from the tumor. Although death has resulted from cardiomyopathy, such fatalities usually occur after the dog otherwise would have died or been euthanized because of its tumor. Concurrent use of IV dexrazoxane (see following section) before doxorubicin administration has a cardioprotectant effect and can be considered in breeds at risk, in dogs that are exceeding the usual cumulative dose cutoff, or in cases involving pre-existing cardiac disease when no other effective chemotherapeutic options exist.

In cats, a cumulative nephrotoxicity appears to be a significant concern.[103] The mechanism of this toxicity remains to be determined, but renal function should be monitored throughout and after doxorubicin therapy in cats.

Dexrazoxane. Dexrazoxane (Zinecard for injection; Pfizer; 250 and 500 mg powder for reconstitution) is a commercially available agent shown to block doxorubicin-induced cardiac toxicity.[102] Dexrazoxane protects against iron-mediated free radical formation but does not interfere with the anticancer effects of doxorubicin because iron-dependent free radical generation is not the main cytotoxic mechanism. The dose of dexrazoxane is based on the dosage of doxorubicin.

The recommended dexrazoxane-doxorubicin ratio is 10:1; that is, 300 mg/m² dexrazoxane to 30 mg/m² doxorubicin. The drug is given as a slow IV bolus starting 30 minutes before administration of the doxorubicin. Because cardiotoxicity is uncommon in dogs and rare in cats, dexrazoxane is not routinely used in veterinary patients. No clinical reports are available on the safety or efficacy of dexrazoxane in cats.

Doxorubicin HCl Liposome Injection. Doxorubicin HCl liposome injection (Doxil; Ortho Biotech Products L.P.; 20 mg vials for injection); compared to other drugs, doxorubicin HCl liposome injection is very expensive and causes significant cutaneous toxicity but does not produce significant myelosuppression or cardiotoxicity compared to native doxorubicin.

Mechanism of Action As its name suggests, this drug is pegylated liposome-encapsulated doxorubicin. SL encapsulation of doxorubicin greatly increases the drug half-life, to more than 40 times that of native drug.[40] Because this drug persists in the circulation longer, it is able to penetrate leaky tumor vasculature more effectively than native doxorubicin.[106] The actual antitumor effect is believed to be the same as with native doxorubicin.*

Dosage and Indications Doxil is dosed in dogs and cats at 1 mg/kg given intravenously every 3 weeks. It must be administered through a perfectly placed IV catheter. Activity in dogs against cutaneous T-cell lymphoma, multicentric lymphoma, hemangiosarcoma, and histiocytic malignancies and in cats against injection site sarcomas has been documented.[40-42]

Toxicity Liposome-encapsulation of doxorubicin significantly reduces the cardiotoxic effects of the native drug. Doxil has not been associated with cardiomyopathy in dogs, even at significantly higher doses than are used with doxorubicin.[107] Like doxorubicin, Doxil may cause irritation if injected perivascularly. Hypotensive episodes have been observed in up to 10% of patients receiving this drug for the first time.[40] In dogs and humans, Doxil also is associated with a cutaneous toxicity syndrome known as palmar plantar erythrodysesthesia (PPES).[41] This syndrome is reported in humans treated with prolonged infusions of various chemotherapeutic drugs. Dogs affected with PPES may develop irritation, alopecia, and ulceration in the axilla, inguinal region, and skin around the footpads (Figure 11-6). The condition is self-limiting, but treatments need to be delayed or discontinued to allow resolution of the affected areas. Co-administration of pyridoxine (vitamin B_6, 50 mg given orally three times a day) reduces by

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*References 30, 32, 40, 42, 94, and 107.

A   B
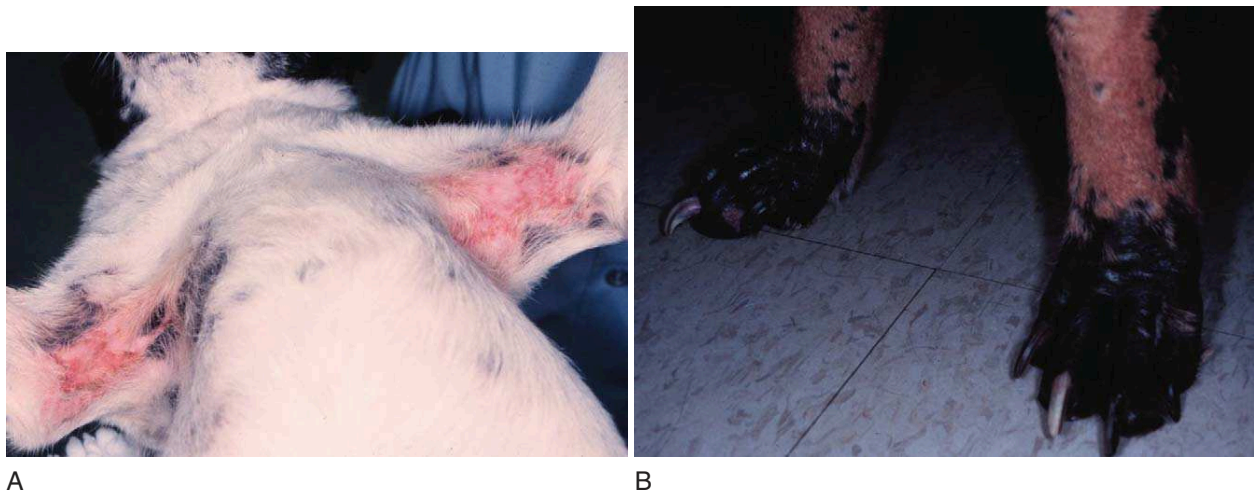
Figure 11-6

A, A dog with palmar plantar erythrodysesthesia (PPES) after three treatments with stealth liposome doxorubicin (Doxil); irritation, alopecia, and ulceration in the axilla can be seen. **B,** Another dog with PPES showing permanent denuding and hyperpigmentation of the paws.

more than fourfold the likelihood of severe PPES in dogs treated with Doxil.[41] Cats may develop focal alopecia on the chin and limbs.[42]

Anthracycline Analogues

Idarubicin and epirubicin are analogs of doxorubicin that were developed with the intent of producing a drug with less cardiotoxicity and equal or greater efficacy.[106] They are included in this chapter because they are commonly used in human medicine as alternatives to doxorubicin; however, limited information is available regarding the efficacy of these agents in veterinary medicine.

Idarubicin. Idarubicin (Idamycin; Pharmacia & Upjohn; 5, 10, and 20 mg vial for injection; 5, 10, and 25 mg capsules for oral administration in Canada only) is a synthetic anthracycline analog that is used to treat acute myelogenous leukemia, lymphoma, myeloma, and breast cancer in humans.[106] The drug may be administered orally or intravenously. In humans, oral absorption is poor at 20% to 30% of the administered dose, and the drug is eliminated through biliary and renal mechanisms. One report exists of the oral use of the drug in cats,[108] and no articles have been published on clinical use of the drug in dogs. For cats, the oral dose is 2 mg/cat/day for 3 days every 3 weeks. Reported toxicities include anorexia, vomiting, and leukopenia. Idarubicin may have activity against lymphoma, but additional clinical trials are needed.

Epirubicin. Epirubicin (Ellence; Pharmacia & Upjohn; 25, and 100 mg vials for injection) is a semisynthetic stereoisomer of doxorubicin. It has the same clinical indications as doxorubicin but is less cardiotoxic.[106] Based on a single case report describing the use of epirubicin in a 10-month-old dog after removal of a uterine carcinoma, a safe dose appears to be 30 mg/m^2 given intravenously every 3 weeks.[109] The dosage and indications for cats are unknown.

Mitoxantrone

Mitoxantrone (Novantrone; Serono; 20, 25, and 30 mg vials that must be diluted before injection) is very expensive and moderately toxic compared to other drugs.

Mechanism of Action Mitoxantrone is a synthetic antitumor antibiotic that inhibits topoisomerase II. It is cell cycle phase nonspecific. Unlike with doxorubicin, free radical formation does not appear to be involved in the cytotoxicity of mitoxantrone.[106] The drug is metabolized primarily through the liver and is eliminated in the urine and feces.

Dosage and Indications Mitoxantrone is given intravenously at a dosage of 5 to 5.5 mg/m^2 in dogs and 6.5 mg/m^2 in cats once every 3 weeks. It should be diluted with up to 50 ml of 0.9% sodium chloride or 5% dextrose for injection. Mitoxantrone has been used in dogs with some success to treat lymphoma, squamous cell carcinoma, and transitional cell carcinoma and in cats as a radiation sensitizer against oral squamous cell carcinoma.[110-114]

Toxicity The side effects of mitoxantrone are myelosuppression, vomiting, and diarrhea. Unlike doxorubicin

it does not commonly cause allergic reactions or tissue damage with extravasation, and it appears to be less cardiotoxic than doxorubicin.[106] In rare cases it may turn the patient's urine blue-green and give the sclera a bluish tint.

Antimetabolites

Cytarabine. Cytarabine (Cytosar-U, cytosine arabinoside, ara-C; Adria, Pharmacia & Upjohn, Bedford Labs; 20, 100, or 500 mg and 1 and 2 g powder for reconstitution); compared to other drugs, cytarabine is inexpensive and well tolerated.

Mechanism of Action Cytarabine is a pyrimidine analog that is cell cycle phase specific. It primarily kills cells undergoing DNA synthesis (S-phase) and under certain conditions blocks the progression of cells from the G_1 phase to the S-phase. Although the mechanism of action is not completely understood, cytarabine appears to act through the inhibition of DNA polymerase. After subcutaneous (SQ) or intramuscular (IM) injection, it reaches therapeutic levels in the CSF. It is extensively metabolized and is excreted in the urine.

Dosage and Indications The dosages of cytarabine for dogs and cats are 150 mg/m^2 given subcutaneously twice daily for 2 days or 600 mg/m^2 given intravenously or subcutaneously once weekly.[115,116] Because this drug is cell cycle specific, a continuous-rate infusion is ideal for maximal cycling cell exposure. However, a more practical administration protocol is to divide the dose as previously described. Indications for cytarabine include lymphoma that affects or has the potential to affect the central nervous system (CNS), such as renal lymphoma in cats, and some leukemias.[115,117]

Toxicity Side effects of cytarabine generally are mild. Thrombocytopenia is the most common side effect; neutropenia, vomiting, diarrhea, and anorexia are rare.[115]

Gemcitabine. Gemcitabine (Gemzar; Eli Lilly; 200 mg and 1 g powder for reconstitution); compared to other drugs, gemcitabine is expensive and well tolerated.

Mechanism of Action Gemcitabine is a deoxycytidine analog that undergoes intracellular activation and results in inhibition of DNA synthesis.[118] It is cell cycle specific.

Dosage and Indications In normal dogs, intravesical administration of gemcitabine at doses of 100 mg, 350 mg, and 1000 mg/m^2 given intravenously appear to cause minimal adverse effects.[119] A safe and well-tolerated dose of gemcitabine in tumor-bearing dogs is 675 mg/m^2 given intravenously as a 30-minute infusion every other week.[118] Anecdotally, gemcitabine appears to be tolerated when administered at a dosage of

300 mg/m^2 given intravenously once weekly 3 weeks out of 4. This drug has not been studied extensively in small animals. Limited efficacy has been reported in tumor-bearing dogs, and results have been disappointing in most cases. Positive responses have been observed in a small number of dogs with lymphoma, oral malignant melanoma, and perianal squamous cell carcinoma.[118]

Use of low-dose gemcitabine (50 mg/m^2 given intravenously twice weekly in dogs and 25 mg/m^2 given intravenously twice weekly in cats) in conjunction with radiation therapy has been reported.[120,121] One group treated cats with oral squamous cell carcinoma with the aforementioned doses and twice weekly palliative radiation.[120] Toxicity attributable to chemotherapy was mild; of eight cats treated, two experienced complete remission and four underwent partial remission. The same study reported the use of gemcitabine at 75 to 115 mg/m^2 given intravenously weekly with no complications in one cat after completion of radiation therapy. However, dogs treated for sinonasal carcinomas with definitive radiation and low-dose, twice weekly gemcitabine developed significant hematologic toxicity (neutropenia) that required dose reductions; 10 of 15 dogs had severe local tissue toxicity that required early discontinuation of radiation.[121] Overall median tumor control was 8 months, and two dogs had no evidence of disease for 20 months or longer. Cats treated with twice weekly gemcitabine and definitive radiation developed moderate to severe neutropenia that resulted in dose reductions, and in six cats radiation therapy had to be stopped early because of severe local tissue toxicity.[121]

Toxicity Gemcitabine as a single agent appears to be well tolerated in dogs and cats; the most common toxicity is mild GI upset.[118] As a radiosensitizer, at the doses described previously, gemcitabine may be associated with severe local tissue toxicity (at the radiotherapy site) and moderate to severe neutropenia.[121] The reason for the discrepancy between the two reports combining gemcitabine with radiation is unclear, but further studies need to be done before this treatment combination can be recommended.

Methotrexate. Methotrexate (Methotrexate tablets, Methotrexate for injection, Rheumatrex Methotrexate Dose Pack; STADA, various pharmaceutical companies, including Pharmacia & Upjohn, Bedford Labs, American Pharmaceutical Partners; 2.5, 5, 7.5, 10, and 15 mg tablets and 20 mg and 1 g powder for reconstitution for IM or IV administration); compared to other drugs, methotrexate is inexpensive and well tolerated at typical doses.

Mechanism of Action Methotrexate inhibits dihydrofolic acid reductase and thus interferes with DNA synthesis, repair, and cellular replication in a cell cycle phase–specific manner. When given orally, methotrexate may be partly

metabolized by intestinal flora. Methotrexate undergoes hepatic metabolism after absorption. Renal excretion is the primary means of elimination, the rate of which depends on the dose and route of administration.

Dosage and Indications The recommended dosage of methotrexate for dogs and cats is 0.6 to 0.8 mg/kg given intravenously or orally. Oral absorption appears to be dose dependent. In veterinary medicine, methotrexate most commonly is used as part of a combination chemotherapy protocol for the treatment of lymphoma.[59,122] However, because of its very short half-life, this drug is not widely used.

Toxicity The primary side effects of methotrexate are gastrointestinal in nature (diarrhea, nausea, vomiting, and anorexia). Leukopenia, thrombocytopenia, temporary hair loss, pyrexia, skin rashes or discoloration, and oral lesions can occur but are not common. At very high doses nephrotoxicity may occur as a result of precipitation of the drug in the renal tubules. In humans, a regimen of high-dose methotrexate (doses 10 times higher than standard doses) followed by "rescue" with folinic acid (a form of tetrahydrofolate) is used to treat some tumor types (e.g., osteosarcoma).

5-Fluorouracil (5-FU). 5-Fluorouracil (5-FU) (Efudex cream or solution, Roche Dermatologics; Fluoroplex topical solution and cream 1%; Adrucil or Fluorouracil injection by Adria, Pharmacia & Upjohn, Gensia Sicor, and others; 500 mg and 1, 2.5, and 5 g vials for injection); compared to other drugs, 5-FU is inexpensive and well tolerated in dogs.

Mechanism of Action 5-FU is metabolized into a nucleotide that is incorporated into RNA and ultimately interferes with DNA synthesis, specifically inhibiting S-phase. After extensive intracellular metabolism, the drug is eliminated through the lungs and kidneys.

Dosage and Indications In dogs, 5-FU may be applied topically with minimal systemic absorption; IV administration results in wide distribution to most tissues, including the CSF. One reported injectable dose is 150 mg/m² given intravenously once weekly.[90,123,124] 5-FU has been used in combination protocols for carcinomas and sarcomas and has shown some limited effect against nasal adenocarcinomas and GI and mammary carcinomas.[90,123,125,126]

Toxicity Side effects in dogs include moderate GI upset, myelosuppression, and the potential for severe neurotoxicity if 5-FU is administered above the recommended dose.[127] Ingestion (usually of the owner's skin cream) of more than 20 mg/kg is associated with toxicosis in dogs, and ingestion of more than 43 mg/kg is fatal.[128,129] 5-FU causes fatal neurotoxicity in cats and should not be used in this species; even topical application may be lethal.[124,128,130]

Antitubulin Agents

The antitubulin agents, particularly vincristine and paclitaxel, are associated in humans with the development of a peripheral neuropathy characterized initially by paresthesias and dysesthesias; loss of motor function can occur with persistent administration of the drug.[131] Rats treated experimentally with vincristine develop disorganization of the axonal microtubule cytoskeleton and an increase in the caliber of unmyelinated sensory axons.[131] Electrophysiologic recordings of these rats show that approximately half of the peripheral nerve C-fiber nociceptors are markedly hyperresponsive to stimulation. Mechanistically, the vinca alkaloids likely affect neuronal microtubules, resulting in impaired axonal transport in peripheral nerves. Vinblastine and, subsequently, vinorelbine were developed as less neurotoxic alternatives to vincristine.

Paclitaxel. Paclitaxel (Taxol; Bristol-Myers Squibb; 30, 100, and 300 mg multidose vials that must be diluted before injection); compared to other drugs, paclitaxel is expensive and poorly tolerated.

Mechanism of Action The taxanes are an important class of antineoplastic agents. They are similar to vincristine and vinblastine in that they are antimicrotubular agents, but the taxanes bind to tubulin by a different mechanism than the vinca alkaloids. Taxanes reduce the critical concentration of tubulin required for microtubule assembly, resulting in a mitotic block at the metaphase/anaphase junction and in cell cycle phase–specific activity. In addition to cytotoxic activity, antiangiogenesis is another mechanism of antitumor activity. Paclitaxel is metabolized by the hepatic P450 microsomal system and is eliminated primarily in the feces.

Dosage and Indications The recommended dosage of paclitaxel for dogs is 132 mg/m² given as a slow IV bolus every 3 weeks; however, this drug is difficult to deliver and is associated with significant toxicity. It should be reserved for committed clients when alternative therapeutics are not available.[132] A protocol for the administration of paclitaxel is presented in Figure 11-7. Although a report exists of *in vitro* efficacy of paclitaxel against feline sarcoma cell lines,[133] there are no reported doses, premedication protocols, or clinical indications for use of the drug in cats. Paclitaxel had an antitumor effect and minimal toxicity when administered by means of inhalation therapy, but this route of delivery is not readily available.[34]

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Date: _____   **Treatment #**: _____

CBC OK: YES   NO

Body Weight:____kg _____ m^2

Pretreatment Protocol:

1) Diphenhydramine (4 mg/kg) IM
   30 to 60 minutes prior to Taxol treatment   ____ time given

2) Cimetidine (4 mg/kg) IV   ____ mg
   30 to 60 minutes prior to Taxol treatment   ____ time given

3) Dexamethasone SP (2 mg/kg) IV   ____ mg
   30 to 60 minutes prior to Taxol treatment   ____ time given

Taxol Protocol: Taxol dose is 165 mg/m^2

4) Taxol dose in mg: ____ mg

5) 0.9% NaCl mixed with Taxol (ml Taxol x 10)   ____ ml

6) Rate of infusion = 30 ml/hr (0.5 ml/min) **If no allergic reaction
   after 30 minutes, increase infusion rate to 1.0 ml/min.

7) Time Taxol infusion began   ____ time

8) Flush IV with 25 to 30 cc 0.9% NaCl when complete

   NB: Observe closely for anaphylaxis and call clinician if observed

Figure 11-7

Taxol administration protocol (intravenous).

Toxicity Paclitaxel may cause significant myelosuppression with the nadir at 3 to 5 days; GI effects, such as vomiting and diarrhea, are uncommon. Hypersensitivity, a more specific toxicity noted with this drug, occurs because of the polyoxyethylated castor oil (Cremophor EL) solution that is required to solubilize the drug. Reactions may be severe, including neurologic and cardiovascular signs. Even with pretreatment with diphenhydramine (4 mg/kg given intramuscularly), cimetidine (4 mg/kg given intravenously), and dexamethasone SP (2 mg/kg given intravenously) 30 to 60 minutes before chemotherapy, most patients still have an allergic reaction.[132] Because of the severity of the hypersensitivity reaction, administration of prednisone (2 mg/kg given

orally) the night before therapy is recommended in addition to the previously described premedication protocol.

Vinblastine. Vinblastine (Velban, Velsar; Eli Lilly, Adria; 10 mg powder for reconstitution or 10 and 25 mg vials for injection); compared to other drugs, vinblastine is inexpensive and well tolerated.

Mechanism of Action Vinblastine is the salt of an alkaloid extracted from a flowering herb commonly known as periwinkle. Vinblastine is cell cycle phase specific; it binds to microtubular proteins in the mitotic spindle, thereby preventing cell division during metaphase. It also interferes with amino acid metabolism by inhibiting glutamic acid utilization and

preventing purine synthesis, citric acid cycle, and urea formation. Vinblastine is extensively metabolized by the liver and is primarily excreted in the feces.[134]

Dosage and Indications The standard dosage of vinblastine for dogs is 2 mg/m^2 given intravenously once weekly or every other week. Currently the authors incrementally increase the dosage by 0.33 mg/m^2, if well tolerated, up to 4 mg/m^2. No published studies cite a therapeutic dose for cats; however, anecdotal experience suggests that most cats tolerate a dosage of 2 mg/m^2 given intravenously once weekly. The frequency of administration depends on the tumor type; vinblastine can be administered weekly with CBC monitoring. Vinblastine is used to treat canine mast cell disease[135,136] and can be used in lymphoma protocols as a substitute for vincristine when a neuropathy is suspected.

Toxicity Vinblastine typically is well tolerated, with rare GI or myelosuppressive side effects. Extravasation of vinblastine may cause significant tissue irritation and cellulitis, therefore the drug must be given through a clean IV puncture.[134]

Vincristine. Vincristine (Vincristine sulfate, Oncovin solution, Vincasar; Eli Lilly, Adria; 1, 2, and 5 mg vials for injection); compared to other drugs, vincristine is inexpensive and well tolerated. It is available as an aqueous solution or in powder form that requires reconstitution. The concentration of the aqueous solution is 1 mg/ml; the solution should be kept refrigerated and protected from light to prevent degradation of the drug. The undiluted powder form can be stored at room temperature for 6 months. Once diluted, the drug may be stored in the refrigerator for 28 days.

Mechanism of Action Vincristine also is derived from periwinkle and also is cell cycle phase specific. Its mechanism of action is through inhibition of intracellular microtubule formation, an important part of the mitotic spindle formation. The initial, middle, and terminal half-lives of the drug are 5 minutes, 2.3 hours, and 85 hours, respectively. Vincristine is metabolized by the liver and eliminated in the feces. Liver dysfunction is a contraindication to the use of vincristine.[134]

Dosage and Indications The dosage of vincristine in dogs and cats is 0.5 to 0.75 mg/m^2 given intravenously as a rapid bolus. Vincristine can be used as a single agent or as part of a multiagent protocol; dosing schedules vary. Some of its uses are for lymphoproliferative cancers, TVTs, and immune-mediated thrombocytopenia (through increased release of platelets from the bone marrow).*

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*References 60, 65, 85, 93, 134, 137, and 138.

Toxicity The major side effect of vincristine is severe perivascular tissue reaction with accidental extravasation. This causes an acute burning sensation and results in tissue sloughing in the affected area. Other possible side effects include constipation, especially in cats, and peripheral neuropathy with prolonged administration. Such neuropathy is rare in veterinary medicine and has been difficult to diagnose definitively, but dogs may show signs of generalized pain and may lick and chew at the digits when affected. Abdominal cramping also has been reported anecdotally. Substitution of vinblastine in such cases has resolved the reactions. Although vincristine has been reported to be less myelosuppressive than other chemotherapeutic drugs, and it is used in combination with other drugs, recent reports have documented moderate to severe myelosuppression after vincristine therapy.[139,140]

Vinorelbine. Vinorelbine (Navelbine; GlaxoWellcome; 10 and 50 mg vials for injection); compared to other drugs, vinorelbine is expensive but well tolerated.

Mechanism of Action Vinorelbine is a second-generation, semisynthetic vinca alkaloid. Its mechanism of action is through binding to microtubular proteins in the mitotic spindle, thereby preventing cell division during metaphase. Vinorelbine is hepatically metabolized and cleared through the feces, therefore the dosage should be reduced in animals with significant hepatic dysfunction.

Dosage and Indications The dosage of vinorelbine for dogs is started at 15 mg/m^2 given intravenously once weekly or every other week.[141] If this dose is well tolerated, incremental increases (3 mg/m^2) in subsequent doses can be used. The drug should be kept refrigerated until the time of administration. The dose to be administered should be diluted 1:5 with sterile 0.9% sodium chloride; the treatment should be given through a cleanly placed IV catheter over 5 to 10 minutes. Although the data are limited, a phase I dose-finding study reported that vinorelbine had a 12.5% PR rate against gross tumors in dogs.[141] Further evaluation of this drug is underway, now that the MTD has been established. Currently, the main indication for vinorelbine is treatment of primary lung tumors; in the phase I trial, two of six dogs with gross bronchoalveolar carcinoma experienced partial responses.[141] Vinorelbine currently is being evaluated as an adjuvant to lung lobectomy in node-positive bronchogenic carcinoma in dogs.

Toxicity In humans, vinorelbine may cause peripheral neuropathy or constipation. In dogs, neutropenia is the dose-limiting toxicity; neurotoxicity appears to be very limited.[141] As are all vinca alkaloids, vinorelbine is a vesicant and therefore must be administered through a carefully placed indwelling catheter. The dosage,

dose-limiting toxicities, and indications for use in cats are unknown.

Hormones

Prednisone. Compared to other drugs, prednisone is inexpensive and moderately well tolerated.

Mechanism of Action Prednisone is a commonly used catabolic steroid that binds to cytoplasmic receptors and inhibits DNA synthesis. Prednisone is converted to the active form, prednisolone, in the liver. Patients with hepatic dysfunction should be treated with prednisolone rather than prednisone.

Dosage and Indications The antitumor dosage of prednisone is 2 mg/kg given once daily by mouth or by infection. This dosage is gradually reduced and the drug eventually is discontinued when it is used in combination protocols.[65,135] Prednisone has antitumor activity against lymphoma, mast cell tumors, and plasma cell tumors.[65,88,135,142] Other indications for prednisone in veterinary oncology include patients with intracranial tumors, insulinomas, or hypertrophic osteopathy.[143,144] At antiinflammatory dosages (0.5 mg/kg/day), prednisone is effective at reducing edema associated with space-occupying lesions in the brain or spinal cord. It also has antiinsulin properties that are useful for maintaining euglycemia in insulinoma patients. In addition, prednisone can reduce the swelling and pain associated with the paraneoplastic syndrome of hypertrophic osteopathy.

Toxicity Use of prednisone may lead to signs of iatrogenic hypercortisolism, including polyuria, polydipsia, polyphagia, hepatomegaly, hair loss, muscle wasting, and panting. Some dogs may show hyperactivity or depression while on steroids. Overall, although these side effects are not life threatening, they may greatly diminish the patient's quality of life. In addition, corticosteroids may cause GI ulceration. They should never be used in combination with nonsteroidal antiinflammatory drugs.

Miscellaneous Agents

L-Asparaginase. L-Asparaginase (Elspar; Merck; 10,000 IU powder for reconstitution); compared to other drugs, asparaginase is moderately expensive and very well tolerated.

Mechanism of Action L-asparaginase is a bacteria-derived enzyme that degrades the amino acid asparagine, depriving growing cells of that amino acid and inhibiting protein synthesis. Unfortunately, many tumor cells are able to increase the activity of their endogenous asparagine synthetase and thus become resistant to the antitumor effects of L-asparaginase. Also, antibodies to the foreign bacterial protein develop after drug exposure, leading to resistance and allergic reactions (see Toxicity section). L-asparaginase is eliminated through the urine and feces.

Dosage and Indications The dosage of L-asparaginase for dogs and cats is 400 IU/kg given subcutaneously or intramuscularly, or 10,000 IU/m² given subcutaneously or intramuscularly. A recent study found that dogs with stage III lymphoma had longer survival times when treated via the IM route compared to the SQ route; however, the number of study subjects was small, and the SQ route is associated with fewer allergic episodes.[145] Animals should be monitored for hypersensitivity reactions for 60 minutes after drug administration. L-asparaginase is used in combination chemotherapy protocols for the treatment of lymphoma. Several studies have confirmed that adding the drug to induction protocols does not result in clinically relevant increases in remission rate, speed of attaining remission, or first-remission duration, therefore it is best reserved for rescue situations.[145-148] Many protocols limit the use of L-asparaginase to avoid the development of resistance.[65,93,149,150]

Toxicity The main potential adverse drug reaction to L-asparaginase is a hypersensitivity reaction, which usually is seen within 60 minutes of administration. Affected animals may show vomiting, diarrhea, urticaria, edema, pruritus, dyspnea, restlessness, hypotension, and rarely collapse (Figure 11-8). The likelihood of these reactions increases with subsequent doses. Management of this reaction includes administration of H₁ blockers (diphenhydramine, 2 mg/kg given intramuscularly), glucocorticoids (dexamethasone SP, 0.5 to 1 mg/kg given intravenously), IV fluids and, if the reaction is severe, epinephrine. Less common toxicities include pancreatitis and myelosuppression. For dogs with known hypersensitivity to L-asparaginase, a pegylated form of L-asparaginase (PEG-L-Asparaginase) is commercially available and has been shown to have reduced toxicity and equivalent therapeutic activity to native L-asparaginase in dogs with lymphoma.[151] Significant myelosuppression may occur when L-asparaginase is combined with vincristine.[140] Although the mechanism for this toxicity is unclear, L-asparaginase may inhibit normal liver function, which may impair hepatic excretion of vincristine. The timing of L-asparaginase administration (i.e., simultaneously with or 12 to 24 hours before vincristine) does not appear to affect toxicity.[140]

Carboplatin. Carboplatin (Paraplatin for injection; Bristol-Myers Squibb, Mayne; 50, 150, and 450 mg vials for injection); compared to other drugs, carboplatin now is relatively affordable and well tolerated (the cost of this drug dropped dramatically in 2005).

A

B
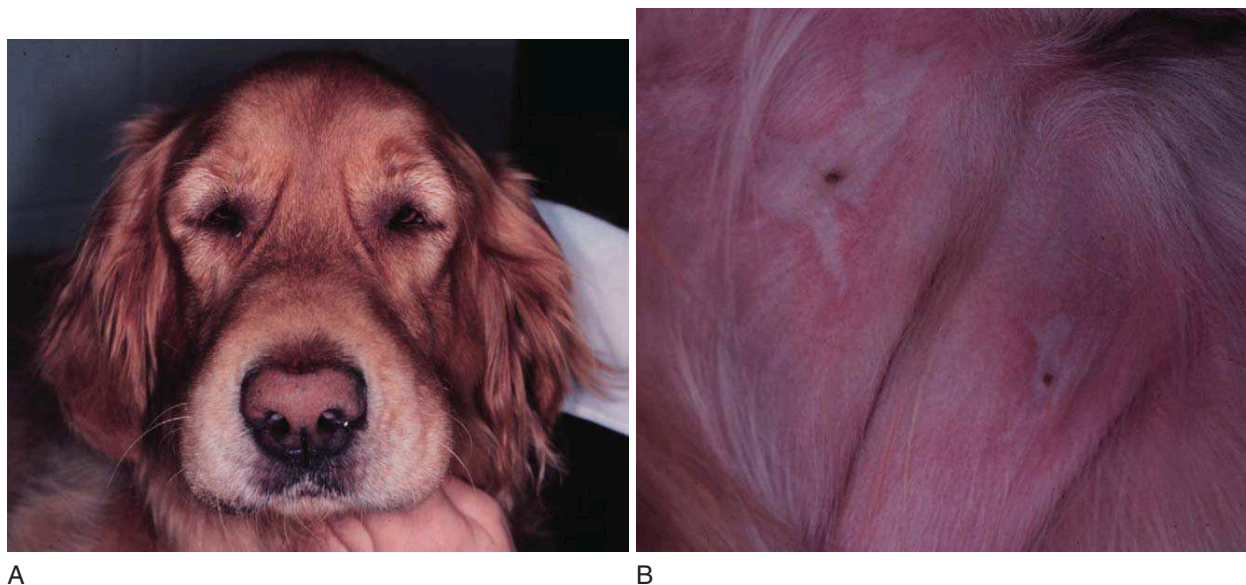
Figure 11-8

A, Facial edema in a dog experiencing an allergic reaction to L-asparaginase. **B,** Urticaria in the same dog in the inguinal region.

Mechanism of Action Carboplatin is a platinum-containing compound. Its mechanism of action is through binding within and between DNA strands, thereby inhibiting protein synthesis in a cell cycle phase–nonspecific manner. Carboplatin is eliminated in the urine.

Dosage and Indications The dosage of carboplatin for dogs is 300 mg/m^2 given intravenously over 5 to 10 minutes every 3 weeks; cats are treated at a dosage of 240 to 260 mg/m^2 given intravenously over 5 to 10 minutes every 3 to 4 weeks. Carboplatin may be used in place of cisplatin to treat dogs with osteosarcoma.[152,153] Its activity has not been well documented against most tumor types, but it is considered a reasonable option for the treatment of various carcinomas and sarcomas.

Toxicity Unlike cisplatin, carboplatin is not emetogenic, and it also can be safely administered to cats.[154,155] Carboplatin is myelosuppressive. Neutrophils and platelets are affected in dogs, with the nadir occurring 11 to 14 days after drug administration.[156] A CBC and platelet count should be evaluated 10 to 14 days after drug administration. In cats, neutropenia is the most common complication, with the lowest counts occurring at 14 to 21 days. A CBC should be done at both time points until a nadir is established.[154] Although not nephrotoxic, carboplatin is excreted by the kidneys and should not be used in an animal with compromised renal function.[157,158]

Cisplatin. Cisplatin (Platinol, Platinol-AQ injection; Bristol-Myers Squibb and various other companies; 50,

100, and 200 mg vials for injection); compared to other drugs, cisplatin is expensive, labor intensive to administer, and moderately toxic.

Mechanism of Action Cisplatin is a heavy metal compound that binds within and between DNA strands, thereby inhibiting protein synthesis. It is cell cycle phase nonspecific. After administration of an IV dose, cisplatin is rapidly bound to plasma proteins. It is eliminated through the urinary tract.

Dosage and Indications Figure 11-9 presents the cisplatin administration worksheet, which gives specific dosages, fluid rates, and antiemetic therapy. Cisplatin is used to treat canine osteosarcoma, squamous cell carcinoma, and various other carcinomas.[159-161]

Toxicity Cisplatin is highly emetogenic; patients often vomit within 1 to 4 hours of its administration. An antiemetic (e.g., single agent or combination butorphanol, prochlorperazine, metoclopramide, ondansetron, or dolasetron) should always be used before cisplatin is administered. Cisplatin is also nephrotoxic and should always be given concurrently with a 0.9% saline-induced diuresis. Cisplatin causes a fatal pulmonary edema in cats and therefore cannot be used systemically at any dosage in this species.[162]

Hydroxyurea. Hydroxyurea (Hydrea capsules, Mylocel; Bristol-Myers Squibb, MGI Pharma and various other companies; 500 mg capsules); compared to other drugs, hydroxyurea is inexpensive and well tolerated.

```
                                                    Date_____

                                        Chemotherapy tx #_____

                                        Weight (kg) _____ m² _____




              Patient ID Imprint


       Obtain: CBC_____ creatinine_____ urine specific gravity_____
       (0.9% NaCl-induced diuresis may be started before getting these results)

  0.9% NaCl infusion @ 18.3 ml/kg/hr IV for 4 hours      time_____ rate_____

  BUTORPHANOL (0.4 mg/kg IM) 1/2 hour before end of saline infusion

                                                 time_____ dose_____

  CISPLATIN (50 – 70 mg/m² IV over 20 minutes) in enough saline to maintain
  the same fluid rate as listed above

                                                 time_____ dose_____

  0.9% NaCl infusion @ 18.3 ml/kg/hr IV for 2 hours      time_____ dose_____

  Do NOT administer cisplatin if neutrophil count is ≤ 2,000 cells/µl, or if creatinine
  is above normal.
```

Figure 11-9

Cisplatin administration protocol.

Mechanism of Action Hydroxyurea inhibits the conversion of ribonucleotides to deoxyribonucleotides through the destruction of ribonucleoside diphosphate reductase. It is an S-phase–specific drug that is metabolized by the liver and eliminated in the urine.

Dosage and Indications The recommended dosage for dogs is 50 mg/kg given orally once daily, or divided twice daily. Cats should be treated with 10 mg/kg given orally once daily. The dosage is reduced to every other day as the disease goes into remission. The main indications for use of this agent are polycythemia vera and chronic myelogenous or basophilic leukemia.[163-167]

Toxicity Myelosuppression and GI upset may be seen, particularly in cats. Sloughing of toenails in dogs may be seen with chronic use.

Procarbazine. Procarbazine (Procarbazine hydrochloride, Matulane; Sigma-Tau Pharmaceuticals; 50 mg capsules, the powder is unstable in water or aqueous solutions); compared to other drugs, procarbazine is expensive and, as a component of the MOPP protocol, moderately well tolerated.

Mechanism of Action and Metabolism The exact mechanism of action is unclear, but procarbazine produces inhibition of DNA, RNA, and protein synthesis. It may act through DNA alkylation and methylation. The drug is metabolized in the liver and excreted in the urine.

Dosage and Indications No information is available on the use of procarbazine as a single agent in dogs or cats. It is used at a dosage of 50 mg/m² daily for 14 days on and 14 days off as part of the MOPP protocol (see Mechlorethamine) for the treatment of relapsed canine lymphoma. Because the drug is supplied in 50 mg capsules, the following recommendations have been published[85]:
- *Dogs over 0.8 m²*: Round off dose to the nearest 50 mg.
- *Dogs over 0.4 m² but under 0.8 m²*: Round off dose to the nearest 50 mg but give every other day on days 1 to 13.

- *Dogs under 0.4 m²*: Use 10 mg compounded capsules and round off dose to the nearest 10 mg daily on days 0 to 13.

Toxicity Toxicities with single agent procarbazine are not known. As part of the MOPP protocol, GI toxicity is the major concern. In one study, anorexia, vomiting, or diarrhea affected 33 of 117 dogs (28%); 15 of these dogs required hospitalization and dose reduction.[85] In the same study, sepsis secondary to neutropenia was reported in five of the 117 dogs (4%).

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Radiation Therapy

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Radiation therapy has been used in veterinary medicine since shortly after the discovery of x-rays by Roentgen in 1895. Alois Pommer, an Austrian veterinarian, published extensively on the irradiation of benign and malignant diseases and established a radiation therapy protocol widely used for many years.[1] Through randomized clinical trials and retrospective studies, the efficacy of radiation therapy for many tumors that occur in animals has been established. Newer imaging technology, such as computed tomography (CT), magnetic resonance imaging (MRI), and nuclear scanning have dramatically improved the localization and staging of tumors, allowing radiation therapy to be administered effectively to a wide variety of tumors. Advances in radiation therapy equipment, including isocentrically mounted megavoltage units, linear accelerators with electron capabilities, intensity-modulated radiation therapy (IMRT), and three dimensional computerized treatment planning permit greater sparing of normal tissues and more precise tumor targeting. These technical advances reduce the treated tissue volume, allowing for dose escalation, which should improve the probability of tumor control.

In addition to technologic improvements, a better understanding of the underlying radiation biology of normal and tumor tissues has allowed the development of improved radiation therapy protocols. Radiation therapy is an effective treatment modality for animal cancer patients, just as it is for human cancer patients. Half of human patients with serious cancers undergo radiation therapy at some point.[2] This useful treatment has been unavailable to many animal patients because of the limited number of veterinary radiation therapy centers. However, in recent years the number of these centers has increased dramatically, and clients who live considerable distances from them frequently are willing to transport their animals for cancer management. More than 60 facilities in North America are actively treating animals with radiation therapy.[3] The Veterinary Cancer Society web site provides a list of these centers (www.vetcancersociety.org/pdf/radiation.pdf).

Most veterinarians will never have a radiation therapy unit in their practice. However, several important considerations can aid the practitioner in determining the best use of this treatment modality in the management of cancer patients. These include (1) decision making in the selection of patients; (2) tumors commonly treated with irradiation; (3) palliative radiation therapy; (4) normal tissue tolerance; (5) basic principles of radiation oncology; and (6) radiation therapy equipment.

DECISION MAKING FOR THE TREATMENT APPROACH

The management of cancer patients is complex, and determining the best treatment modality or combination of modalities can be challenging. With most solid tumors, two issues must always be addressed: loco-regional tumor control and control of potential metastatic disease. For local tumor control, treatment options generally include surgery or radiation therapy or both. For loco-regional disease, radiation therapy alone or combined with surgery may be best. This discussion focuses on the principles used to determine whether surgery, radiation therapy, or a combination of these is the most appropriate method of gaining local or loco-regional control of solid tumors. If a tumor is likely to metastasize, adjuvant chemotherapy must also be considered if it has been shown to have antitumor activity for that particular cancer.

Five factors must be considered for every patient in the decision to treat with surgery alone, radiation alone, or a combination of the two:

1. The owner's wishes, commitment, and financial resources
2. The probability of local or loco-regional tumor control
3. The predicted functional outcome
4. The predicted cosmetic outcome
5. Other, concurrent health problems

First and foremost, the owner's wishes must be followed. The veterinarian has an obligation to provide

accurate information about alternatives and the benefits and risks of those alternatives, including the probability of tumor control and complications. Owners must make decisions with which they are comfortable, and those decisions must be respected. Owners must be able to judge fairly in the context of their level of commitment to the time, effort, and financial requirements involved.

If a tumor can be excised with a very good to excellent probability of tumor control, good function of remaining organs and tissues, and good cosmetic outcome, then surgery should be the treatment method selected. Local tumor control can be obtained with surgery alone in many types of animal cancers, including mammary tumors, some oral tumors, some soft tissue sarcomas of the trunk and extremities, and some mast cell tumors, with excellent functional and cosmetic outcomes.

Sometimes local tumor control can be achieved with surgery alone but with impairment of the functional or cosmetic outcome. For example, a dog with a large, bilateral acanthomatous epulis of the maxilla that extends from the incisors caudally to the level of the second premolar would have an excellent probability of tumor control with en bloc surgical resection; however, the functional and cosmetic outcomes may be compromised. Radiation therapy alone has an excellent probability of tumor control with this type of tumor, and the functional and cosmetic outcomes may be improved over those seen with en bloc surgical excision. Informed owners can weigh these factors, as well as cost differences, in selecting the treatment for their pets.

Other factors that must be considered are the patient's overall health and whether it has concurrent diseases. The risk posed by anesthesia sometimes is cited as a disadvantage of radiation therapy; however, modern anesthetic agents and equipment and the use of skilled personnel have greatly reduced this risk. Severe concurrent diseases that drastically reduce the patient's life expectancy may be a factor in determining which treatments can be considered.

The decision to combine radiation therapy and surgery is based on improving tumor control or improving the functional and/or cosmetic outcome. For example, extensive soft tissue sarcomas of the extremity often cannot be controlled with surgical excision alone or with radiation therapy alone. Tumor control can be achieved with amputation, and if local tumor control were the only issue, amputation would be the best treatment alternative. However, by combining surgical excision that reduces the cancer to subclinical (i.e., microscopic) disease with radiation therapy, excellent tumor control can be achieved without compromising the functional and cosmetic outcomes. Ideally, a consultation involving the client, the surgeon, and the radiation oncologist should be held before any therapeutic intervention is provided. Depending on the tumor type and location, sequencing the radiation therapy *before* the surgery may be advantageous. If the patient is to undergo surgical cytoreduction before irradiation, the surgical goals need to be discussed so that the radiation dose prescription and treatment planning are appropriate for the specific surgical procedure.

TUMORS COMMONLY TREATED WITH RADIATION THERAPY

Oral Tumors

Many oral tumors (see Chapter 21, Section A) are responsive to radiation therapy. The region is anatomically complex, and aggressive surgery often can leave functional and cosmetic abnormalities. Acanthomatous epulides, previously called adamantinomas, are very radiation responsive. Tumor control with radiation therapy can be close to 90%.[4] A relationship has been demonstrated between tumor (T) stage and local control.[5] The reported 3-year, progression-free survival (PFS) for T_1 tumors (less than 2 cm) and T_2 tumors (2 to 4 cm) is 86%; it is only about 30% for T_3 tumors (over 4 cm). In both studies a coarse fraction scheme was used.[5] Smaller doses per fraction and increased total doses quite possibly could have resulted in greater local tumor control and fewer complications in all stages, including T_3.

In 2004 a retrospective study of 57 dogs with epulides that were treated with irradiation reported that the overall median time to first event and overall survival were 1210 and 1441 days, respectively.[6] Dogs younger than 8.3 years old (the median age in the study) had a significantly longer median survival time (2322 days) than dogs older than 8.3 years (1106 days). Dogs that received doses higher than 40 Gray (Gy) had significantly longer survival times than dogs that received 40 Gy or less (2994 days versus 143 days). A previous publication by one of the authors of this study had implied that epulides treated with radiation transform into malignant epithelial tumors, such as squamous cell carcinoma.[4] However, the 2004 study refuted that conclusion, primarily because most radiation-induced tumors are mesenchymal in origin.[6] It seemed more plausible that the epithelial tumors identified in the earlier paper originally had been misidentified as epulides. Other oral carcinomas are not as radiation responsive as epulides, and the authors hypothesized that the "transformations" probably were recurrences of tumors that originally were carcinomas. Oral squamous cell carcinomas (SCCs) in dogs have been studied frequently. One study involved SCC tumors in stages T_2, T_3, and T_{2b} (with bone invasion) and T_{3b}. A coarse fractionation protocol of 10 4.5-Gy

fractions resulted in tumor control at 1 year of about 75%.[5,7] Although no serious long-term complications were observed, the statistical prediction of frequency of bone necrosis was 15%. Use of more radiobiologically sound fractionation schedules and more advanced treatment planning could yield a greater tumor control probability. The prognosis for dogs with oral SCC is site dependent. Tumors located more rostrally have a better probability of control.[8] Tumors of the base of the tongue and tonsil are highly metastatic and are likely to recur locally or regionally. In these cases radiation therapy has an advantage over surgery because it includes associated lymphatic structures in the treatment field.

Oral fibrosarcomas are unlikely to metastasize but can be difficult to control locally. The histologic appearance can be deceptive; tumors diagnosed as fibromas or low-grade fibrosarcomas can be extremely aggressive locally.[9] If clinical evidence of rapid growth, invasion into bone, or tumor recurrence exist, the tumor should be treated aggressively in spite of more benign pathologic features. En bloc surgical resection often is curative but difficult to accomplish because of the invasive nature of the tumor. Oral fibrosarcomas are less radiosensitive than epulides and SCC, although tumor control probabilities ranging from 33% to 67% at 1 year have been reported.[10,11] One study of oral tumors reported a PFS at 1 year of 72% for SCC of all T stages and 76% for fibrosarcomas.[12] At 3 years the PFS was 55% for SCC and 40% for fibrosarcomas.

Currently, surgical resection followed by radiation therapy is believed to offer the best probability for tumor control of sarcomas of the oral cavity. Surgical cytoreduction improves the probability of tumor control by radiation therapy by reducing the number of clonogenic cancer cells. Making the decision before surgery to follow it with irradiation can be advantageous. The surgeon then can focus on removing clinical disease (avoiding extensive local dissection, which increases the radiation field size) and obtaining a tension-free closure. The surgery can be less aggressive and more cosmetic when subclinical disease is to be managed with radiation; however, it is extremely important that all macroscopic tumors be removed.

The optimal time to begin irradiation after surgery has not been determined. If difficulty in obtaining primary wound healing appears likely, radiation can be delayed. However, any therapeutic gain from combining modalities will be lost if the tumor recurs in the interim. Initiating radiation therapy immediately after surgery does not appear to be a problem if the suture line is tension free and well vascularized. Forrest and colleagues[13] reported a median survival time of 540 days in eight dogs with oral sarcomas treated with surgery followed by irradiation. This was a significantly shorter period than in dogs with sarcomas

in other parts of the body. The fractionation schedule and total dose were not reported for this specific subpopulation. In tumors too large to be surgically resected to a subclinical level, radiation therapy alone is indicated; however, the probability of long-term tumor control is low.

In cats, oral SCC has a very poor prognosis.[14] Combining curative-intent radiation with etanidazole or mitoxantrone therapy has resulted in median survival times of 116 to 170 days.[15,16] In one study, seven cats with mandibular squamous cell carcinomas that were treated with hemimandibulectomy and mandibular node excision followed by radiation therapy had a median survival time of 420 days.[17] Although the numbers are limited, mandibular squamous cell carcinomas may have a better prognosis than sublingual SCC. Recent studies have indicated that palliative (coarsely fractioned) radiation therapy, with or without chemotherapy, for cats with oral SCC is of dubious value.[18,19] Although radiation is likely to play a role in the treatment of this disease, new approaches are indicated. Radiosurgery or IMRT may be useful approaches to explore.

Oral malignant melanoma in dogs is associated with a high rate of regional and distant metastasis.[20,21] Until an effective chemotherapeutic regimen to address metastatic disease is identified, the role of radiation is to achieve tumor control durable enough for the patient to succumb from metastatic disease rather than local or regional recurrence. In one study, 38 dogs with oral melanoma without evidence of metastasis were treated with 48 Gy delivered in 4 Gy fractions on a Monday-Wednesday-Friday schedule.[12] The median PFS was 17.8 months for all dogs and was stage dependent; for T_1 tumors, it was 38 months; for T_2 tumors, 11.7 months, and for T_3 tumors, 12 months. Metastasis was the first event in 14 of the dogs.

A study of 140 dogs evaluated a different subpopulation in which most of the dogs had regional or distant metastasis at the time of presentation.[22] The dogs were treated either with palliative radiation therapy (coarse fractions; 9 to 10 Gy weekly fractions to a total dose of 30 to 36 Gy) or with more conventional radiation therapy (2 to 4 Gy fractions to as high as 45 Gy or more, with or without surgery and/or chemotherapy). The median times to first event and survival were 5 and 7 months, respectively. Tumor recurrence was the first event in only 27% of the dogs, with new metastases or death accounting for the other 63%. Rostrally located tumors and subclinical tumor volumes were associated with an improved time to first event and survival, whereas the presence of radiographically discernible lysis was associated with a poor outcome. Differences in treatment groups (palliative vs. conventional) could not be discerned, possibly because early metastatic disease masked potential differences in local tumor control.

In a retrospective study of 39 dogs with incompletely resected oral melanoma treated with coarsely fractionated radiation therapy plus platinum-based chemotherapy, the median survival time was 363 days.[23] The dogs received 6 weekly fractions of 6 Gy, with cisplatin (10 to 30 mg/m^2) or carboplatin (90 mg/m^2) administered 1 hour before irradiation. Fifteen percent of the dogs failed locally, and the median time to metastasis was 311 days. Although optimal radiation protocols have not been identified, melanomas may have a low α/β ratio, making them more responsive to coarse fractionation protocols than other tumor types.[24]

Nasal Tumors

Nasal tumors (see Chapter 22, Section D) in dogs are difficult to control. Surgery, chemotherapy, cryosurgery, or immunotherapy alone does not appear to improve survival over no treatment.[25-29] An exception to this is reported in a recent small study by Langova and colleagues,[29b] who evaluated a combination chemotherapy protocol of doxorubicin, carboplatin, and oral piroxicam in eight dogs with advanced nasal tumors. A clinical response rate of 75% was observed, including four complete responses, confirmed by CT imaging. All the dogs experienced resolution of clinical signs after one or two doses, and the protocol was well tolerated. These preliminary results are favorable; however, the case number was small, and more dogs need to be treated in this manner to confirm these findings.

Radiation therapy provides the best reported tumor control for canine nasal tumors and likely needs to be part of any curative-intent treatment regimen. However, approaches for more durable tumor control are needed. Recently radiation potentiators/sensitizers have been evaluated, and the addition of surgery has been revisited (see Chapter 22, Section D). Canine nasal tumors are challenging to treat because they are anatomically complex; they wrap around the cribriform plate and extend caudally down the nasal pharynx. The geometry of the nasal cavity is problematic because the tumor is larger caudally than rostrally, making it difficult to achieve even dose distribution. Tumors in this location may benefit from the emerging radiation therapy technologies, such as tomotherapy (Figure 12-1) and IMRT.[2]

Megavoltage radiation therapy alone has been reported to provide a median survival time of about 1 year.[30-32] In general, chondrosarcomas have the longest response duration. Earlier publications did not identify an advantage in combining megavoltage radiation therapy with surgery.[27,33] However, a recent publication by Adams and colleagues[34] reported a median survival time of 47.7 months in a small group of dogs that underwent surgery after radiation therapy. A CT examination was performed 6 weeks after radiation therapy, and if the tumor had not regressed at least 80%, surgical excision (by means of dorsal rhinotomy) was performed. Late effects from this specific protocol were significant but acceptable to most of the clients; nine of 13 dogs developed rhinitis, and four progressed to osteonecrosis. More aggressive antimicrobial support may improve these effects. Surgery and coarse fractionation reduce normal tissue tolerance to radiation, which contributed to the high percentage of patients with late effects in this study. Nonetheless, administering radiation before surgery may provide a radiobiologic advantage. Preoperative radiation using a protocol better tolerated by normal tissues (i.e., smaller doses per fraction) should be further evaluated.

Surgery generally has preceded radiation therapy when orthovoltage radiation is used, primarily because orthovoltage lacks sufficient penetration to provide a uniform dose in unresected nasal tumors. Although median control rates of 18 to 23 months have been reported, the lack of computerized treatment planning capability for orthovoltage irradiators limits the future of this treatment.[35,36]

The role of chemotherapy combined with radiation therapy for nasal tumors is still unclear. Studies using conventional or low-dose chemotherapy have not improved the outcome over radiation alone.[27,32] Radiation therapy combined with slow-release cisplatin in a small group of dogs significantly improved survival compared with a group of historical controls with sarcomas and carcinomas treated with radiation therapy alone.[37] To administer the cisplatin, an open-cell polylactic acid polymer impregnated with the drug was implanted intramuscularly at a distant site. The median survival time for 14 dogs in the combined radiation-cisplatin protocol was 580 days, compared to 325 days for the 13 dogs in the radiation only group. In a follow-up study of 51 dogs treated with the same radiation-cisplatin regimen, the median survival time was 474 days.[38]

Lymphoproliferative nasal tumors in cats respond well and durably to radiation therapy (these are addressed later in the chapter in the section on lymphoma; also see Chapter 31).[39] Carcinomas and sarcomas in cats respond comparably to nasal tumors in dogs.[40] Cats with nasal tumors treated with 48 Gy administered in 4 Gy fractions over 4 weeks had a 1-year survival rate of 44.3% and a 2-year survival rate of 16.6%. The histologic type and clinical stage of the tumor did not affect the prognosis.

The response to nasal planum SCC is affected by the tumor type and stage.[41] Cats with T_1 tumors had a 1-year survival rate of 85% and a 5-year survival rate of 56%, and the mean was 53 months (the median was not calculable). However, larger, more invasive tumors showed a less favorable response when treated with 40 Gy in 4 Gy fractions over 3.5 weeks. Tumor control should be improved by reducing the dose per fraction and increasing the total dose.
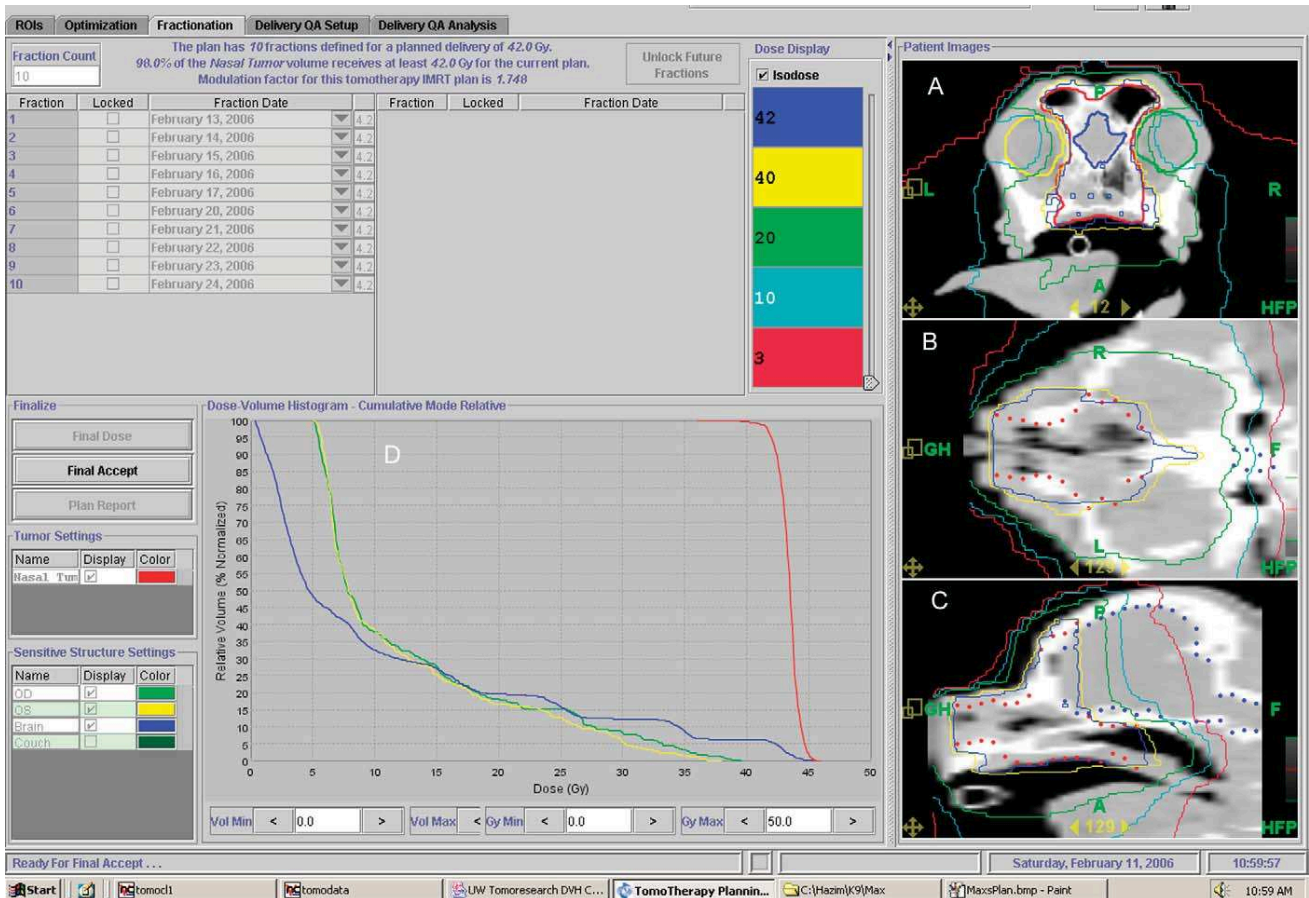
Figure 12-1

Radiation tomotherapy treatment plan for a brachycephalic dog with a nasal tumor. On the right side of the plan are sculpted isodose curves from the transverse plane **(A),** dorsal plane **(B),** and sagittal plane **(C)**. The middle section of the plan **(D)** presents a dose-volume histogram in which red represents tumor volume, green is the right eye, yellow is the left eye, and blue is the brain. (Courtesy Lisa Forrest.)

Brain Tumors

Brain tumors can be treated successfully with radiation therapy (see Chapter 29). In one study, 46 dogs with brain tumors that initially had shown neurologic signs were treated with radiation alone.[42] The median overall survival time was 23.3 months; 69% of the dogs survived 1 year, and 47% survived 2 years. The outcome in this study was superior to those from previous reports, in which the median survival time was about 1 year. No prognostic clinical factors (e.g., tumor size or location or clinical signs) were identified.[43-45] Differences may be due to improved treatment planning capabilities.

Early attempts to treat brain tumors with irradiation were hindered because the treatment machine could not produce small treatment volumes. The radiation tolerance is lower when the entire brain is treated; this makes it impossible to prescribe a dose that is adequate for tumor control but still has an acceptable probability of late effects. Newer accelerators can accommodate smaller fields, and three dimensional (3D) treatment planning systems allow more versatility for sparing normal tissue structures in the region, providing great potential for an improved outcome. In the treatment of brain tumors, surgery is clearly indicated to relieve life-threatening clinical signs. Adjuvant radiation is indicated in patients with incomplete surgical resection. Radiation therapy alone should be performed in dogs with cancer at surgically inaccessible sites or in locations where surgical morbidity is high. In cats, radiation is an effective option for inoperable or incompletely resected tumors.

Radiation therapy should be used in dogs and cats with pituitary macroadenomas. Dogs with pituitary tumors have been reported to have median survival times varying between 1 and 2 years.[46,47] These tumors generally are responsive to radiation, and surgical access is limited. Pituitary irradiation in dogs is more effective at delaying tumor growth than in controlling adrenocorticotropic hormone (ACTH) secretion. Eucortisolism is seen in some patients after irradiation; however, pre-ACTH and post-ACTH cortisol levels should be monitored at regular intervals so that medications can be modified, if indicated.

Cats seem to have marked clinical improvement of associated endocrinopathies. In one study, eight cats with pituitary tumors were treated with radiation therapy, and neurologic signs improved within 2 months in all cats.[48] Endocrinopathies, including hyperadrenocorticism, acromegaly, and insulin-resistant diabetes, were dramatically improved. The median survival time, regardless of cause of death, was 17.4 months. The median survival time was not reached when cats were censored for deaths not caused by the tumor or tumor treatment. Cats with adenomas had longer survival times than those with carcinomas, although the small number of patients precluded meaningful analysis.

The radiation tolerance of brain and spinal tissues generally is considered to be less than that of other commonly treated tissues, and volume may be an important factor for brain and spinal lesions. *Early delayed effects* can occur 1 week to 3 months after treatment and may be due to transient demyelination. Animals with early delayed effects may have signs similar to those of the initial presentation, or they may be generally stuporous. Early delayed effects occur in up to 40% of humans undergoing brain irradiation; symptoms include headache, lethargy, and exacerbation of focal neurologic signs.[49] In animals, clinical signs often are transient, but response time can be slow. Sometimes administration of systemic cortisone and aggressive supportive care are required. CT and MRI may show an apparent increase in tumor size and tumor enhancement during this time. Focal enhancement in normal brain associated with edema and demyelination may also be present.[50]

Late delayed effects probably occur in veterinary patients more often than identified and should be considered as a differential diagnosis for signs that occur during that interval. Late delayed effects (more commonly referred to simply as *late effects*) generally occur at least 6 months after treatment but can also occur years later. Late delayed effects are associated with brain necrosis. The probability of late brain effects depends on the total dose, the fraction size, and the volume of brain irradiated. The signs are similar to those associated with early delayed effects, although the response to steroids is limited. Clinically distinguishing between late effects and tumor recurrence often can be difficult. CT or MRI evaluation can be misleading. Not all brain tumors completely recede after treatment, therefore the presence of a mass does not always indicate a recurring tumor. A prudent course is to obtain a CT or MRI evaluation 6 months after treatment to serve as a reference if clinical signs develop in the future. In one study, three dogs with brain tumors were treated with radiosurgery using a stereotactic head frame.[51] This technology can reduce the tumor volume treated; yet, good biologic principles still must be followed. Administration of radiation in single fractions may be appropriate for palliative treatment of brain tumors; however, long-term tumor control can be better achieved by fractionating radiosurgery or by using radiosurgery as a boost combined with conventional fractionated radiation therapy. The use of dose-volume histograms should help establish defined dose guidelines for the treatment of brain tumors.

Tumors of the Trunk and Extremities

Many tumors involving the trunk or extremities are amenable to treatment by radiation therapy. Small tumors that can be completely excised generally are treated with surgery, although radiation also is effective. Combining radiation therapy with surgery enhances tumor control and improves the functional outcome better than surgery or radiation alone. For tumors in nonresectable locations, radiation alone provides a good outcome. The surgeon and radiation oncologist should consult before therapeutic intervention is started to develop an overall treatment approach.

Hemangiopericytomas, fibrosarcomas, neurofibrosarcomas, myxosarcomas, and nerve sheath tumors are classified together as soft tissue sarcomas because of their similar biologic behavior (see Chapter 20). Metastases are uncommon with grade 1 and grade 2 sarcomas, therefore local tumor control is the primary concern. Soft tissue sarcomas are locally invasive, and tumor cells may extend far beyond the bulk of the tumor. Surgery alone is curative *if* the tumor can be removed completely.[52] If surgical resection is attempted, the margins should be closely examined by a pathologist for evidence of tumor infiltration. If tumor cells extend out to the margin, radiation therapy should be recommended if further resection is not possible. If it is apparent that a tumor cannot be excised completely, treatment combining radiation and surgery can be beneficial. Radiation therapy can be administered first with the hope of converting an inoperable tumor into an operable one. This approach has the benefit of reducing the volume of normal tissues irradiated. As an alternative, surgical excision can be performed first as a cytoreductive procedure and then followed by radiation therapy to kill the residual subclinical disease. In one

study, 48 dogs with soft tissue sarcomas were treated with surgical cytoreduction followed by radiation therapy; only eight dogs (16%) developed tumor recurrence, and the 5-year survival rate was 78%.[53] In a different study, which involved 38 dogs with soft tissue sarcomas of the body and extremities, treatment with surgery followed by irradiation provided a median survival time of 2270 days.[13]

Soft tissue sarcomas can be treated with radiation therapy alone; however, tumor control is not as durable as with a combination of radiation therapy and surgery.[11] Radiation therapy alone is useful for tumors near the pads, where surgical options are limited. Pads in the irradiation field initially may slough; however, if appropriate fractionation schemes are used, the pads regrow and can function normally. Local hyperthermia (see Chapter 15, Section B) can be added to radiation therapy to improve local tumor control in canine soft tissue sarcomas.[54] Adjuvant chemotherapy has been considered for grade 3 soft tissue sarcomas because of the higher metastatic potential of these tumors; however, definitive evidence of improvement is lacking.

Cutaneous mast cell tumors (grades 1 and 2) can be treated successfully with radiation therapy (see Chapter 19). The obvious advantage is that greater margins can be obtained with radiation than with surgery. The probability of control may be improved if surgical cytoreduction is performed first. In a study involving 40 dogs with grade 2 tumors treated with cytoreduction and radiation therapy, tumor control at 1 and 2 years exceeded 90%.[55] In 56 dogs with incompletely resected mast cell tumors, the medium disease-free interval was 32.7 months.[56] Radiotherapy also is indicated for cutaneous mast cell tumors with regional lymph node metastasis. In a study, 19 dogs with mast cell tumors and regional node involvement were treated with surgical cytoreduction of the primary site, radiation to the primary tumor and regional node, and prednisone.[57] The median disease-free survival time was 1240 days. Palliative radiation therapy is commonly used to treat loco-regional mast cell tumors in dogs when systemic spread has occurred.

Vaccine-associated soft tissue sarcomas are a significant problem in cats (see Chapter 20). These tumors are challenging to control locally and seem unresponsive to aggressive radiation therapy or conservative surgery alone. In one study, 33 cats with histologically confirmed fibrosarcomas were treated with radiation therapy followed by surgery.[58] The median disease-free interval and the overall survival time were 398 and 600 days, respectively. In another study, 25 cats with subclinical disease after surgery were treated with radiation therapy alone (57 Gy delivered in 3 Gy fractions) and in some cases with adjuvant chemotherapy.[59] The overall median survival time was 701 days. Recurrence was seen in seven cases (5 of 18 [27.8%] in the group

treated with doxorubicin and 2 of 7 [28.6%] in the group not treated with doxorubicin). In the recurrence cases, one tumor developed outside the treatment field and the others arose in the area treated with radiation. Metastases to the lung or other sites were not seen in this group of cats. Similar findings were evident in 78 cats treated with surgical cytoreduction followed by radiation.[60] In this study, cats that underwent only one surgery before radiation had a lower recurrence rate than cats that had more than one surgery. The survival time and the disease-free interval shortened as the time between surgery and the start of radiation therapy lengthened.

Cats with surgically nonresectable disease present a greater challenge. Escalation of the radiation dose by delivering the dose in smaller fractions probably is necessary for these patients. Also, more sophisticated, 3D treatment planning systems or IMRT may be beneficial for obtaining adequate dose to the tumor, because appropriate sparing of the lungs, viscera, and spinal cord is critical in these patients.

Although osteosarcomas are not considered highly radiation-responsive tumors, radiation may be considered as part of a multimodality therapy when surgical excision is not an option. (Radiation therapy for osteosarcoma is discussed at length in Chapter 23.) Radiation therapy can be combined with chemotherapy and surgery for limb-sparing protocols.[61,62] In a retrospective study of multimodality therapy for axial skeletal osteosarcoma, dogs that underwent curative-intent radiation protocols had a longer duration of tumor control (265 days) than those treated with a palliative regimen (79 days).[63] In another study, stereotactic radiosurgery was performed on 11 dogs with appendicular osteosarcomas.[88] The results are difficult to interpret because the study was a feasibility study in which the radiation dose varied, and not all dogs received adjuvant chemotherapy. However, good limb function and tumor control were observed in some of the patients, which suggests that further evaluation of the technique is warranted.

Other Tumors

Radiation therapy is used for a variety of tumors in the thoracic and abdominal cavities (Table 12-1). The principles of patient selection for radiation therapy with tumors in these regions are the same as for any other region. Radiation therapy must be considered for any tumor that cannot be excised completely. In one study, dogs with thyroid carcinomas treated with 48 Gy delivered in 4 Gy fractions had PFS rates of 80% at 1 year and 72% at 3 years.[64] Thymomas are radiation responsive in human patients. In a study of seven cats with thymoma that were treated with radiation therapy, the median survival time was close to 2 years (see Chapter 32, Section B).

TABLE 12-1	Tumors Commonly Treated with Radiation Therapy			
Tumor Location/Type	**Treatment Modality**	**Control or Survival Data**	**Comments**	**References**
Brain tumors				
Brain tumors, various types and locations—dogs	Radiation	Median survival: 23.3 mo		42
Pituitary macroadenomas and carcinomas—dogs	Radiation only	Median survival: 1-2 yr	For control of neurologic signs.	46, 47
Pituitary macroadenomas and carcinomas—cats	Radiation only	Overall median survival: 17.4 mo, but tumor-related median not reached	May produce profound improvement in endocrinopathies.	48
Tumors of the extremities and body—dogs				
Soft tissue sarcomas	Radiation only	1-Year control: 67% 2-Year control: 33%		11
	Surgery plus radiation	Median control: 86% Median survival: 5-6 yr	Conservative surgery followed by radiation.	13, 53
Mast cell tumors	Radiation only	1-Year control: 44%-78%	Control at 1 year similar to more durable times.	91, 92
	Surgery plus radiation	1-Year control: about 90% Median disease-free interval: 32 mo	For grade 2 tumors.	55, 56
	Surgery plus radiation	Disease-free survival: 3.4 yr	Primary tumor with regional node involvement.	57
Ceruminous gland tumors—dogs and cats	Surgery plus radiation	1-Year control: 56% Mean control: 39.5 mo		90
Tumors of the extremities and body—cats				
Vaccine-associated sarcomas	Radiation followed by surgery	Median disease-free interval: 13 mo Median survival: 18 mo		58
	Surgery followed by radiation	Median survival: 23 mo		59
Lymphoma (localized)	Radiation ± chemotherapy	Median survival: 28 mo	Nonnodal, localized forms.	76
Nasal tumors—dogs				
All types	Orthovoltage radiotherapy plus surgery	Median control: 23 mo	Surgery must be performed for cytoreduction when orthovoltage is used.	35, 36
Carcinomas (all)	Megavoltage radiation therapy	Median control: 8-12.8 mo	The role of adjuvant chemotherapy has not been determined. Preoperative surgery does not appear to improve tumor control.	30, 32, 33, 93
Adenocarcinomas	Megavoltage radiation therapy	Median control: 10-12.8 mo		

TABLE 12-1   Tumors Commonly Treated with Radiation Therapy—cont'd

Tumor Location/Type	Treatment Modality	Control or Survival Data	Comments	References
Nasal tumors—dogs—cont'd				
Squamous cell tumors	Megavoltage radiotherapy	Median control: 6.1-12.8 mo		
Sarcomas (all)	Megavoltage radiotherapy	Median control: 8-12.8 mo		
Chondrosarcomas	Megavoltage radiotherapy	Median control: 12-15 mo		
Carcinomas and sarcomas (all)	Megavoltage radiotherapy	Median control: 16-19 mo	Slow-release-cisplatin–impregnated polymer used for sensitization.	37, 38
Nasal tumors—cats				
Lymphoma	Orthovoltage or megavoltage radiotherapy	Median survival: 20.8 mo		77
Sarcomas and carcinomas	Megavoltage radiotherapy	Median survival: 11.5 mo		39
Oral tumors—dogs				
Acanthomatous epulis	Radiation only	1-Year survival: 85% Median: 48 mo		4, 5
Squamous cell carcinoma	Radiation only	1-Year survival: 65%		8, 12, 94
Fibrosarcoma	Radiation only	Median control: 4 mo	Surgical cytoreduction followed by radiation therapy may improve tumor control.	10, 11
Melanoma	Radiation ± surgery and chemotherapy	Median survival: 8-10 mo	A variety of fractionation schedules. Metastatic disease is a major obstacle to survival.	12, 22, 95, 96
Osteosarcoma—dogs				
Extremities	Radiation (palliative)	Median survival: 4 mo	Provides pain relief.	83
Axial skeleton	Radiation ± surgery and chemotherapy	Median survival: 4.5 mo	Surgery is performed for cytoreduction if paralysis and/or severe pain are present; cisplatin is used as a radiosensitizer.	63, 82

Perianal adenocarcinomas and anal sac adenocarcinomas (see Chapter 21, Section H) can be difficult to control locally with surgery alone and are likely to spread to regional lymph nodes. One hundred and thirteen dogs with carcinomas of the apocrine gland were treated with surgery, radiation therapy, chemotherapy or multimodal treatment. Overall median survival was 544 days. Dogs treated with chemotherapy alone had significantly shorter survival than those receiving other treatments, while the addition of surgery was beneficial.[66] Late effects from radiation therapy to the pelvic region can occur and be clinically significant.[67] This can be addressed by administering the radiation in smaller doses per fraction. Perianal gland tumors are generally slow to disseminate systemically, so full-course treatment of involved regional nodes may be warranted. Intraoperative therapy

as a boost to external beam radiation therapy may also be useful in the treatment of the regional nodes or associated tumor bed following excision.

Lymphoma

Radiation therapy can be important in the treatment of localized lymphoma, and it has an emerging role as an adjuvant therapy in the treatment of systemic lymphoma. Lymphocytes are exquisitely radiation sensitive[68] and may undergo apoptosis in addition to classic mitotic death after exposure to radiation.[69] Human patients with non-Hodgkin's lymphoma commonly receive combined modality treatment with radiation therapy and chemotherapy, primarily for stage I or stage II disease,[70] although this treatment may have a role in more advanced stages as well.[71] Combined modality treatment or radiotherapy alone also is beneficial in humans for primary lymphomas of bone, cutaneous B-cell lymphoma, and mycosis fungoides.[72-75]

In one study, a series of feline extranodal lymphomas were treated with megavoltage radiotherapy with or without concurrent chemotherapy.[76] Involved sites were the nasal cavity, retrobulbar area, mediastinum, subcutaneous tissue, maxilla, and mandible. Eight of 10 cats attained a complete local remission; one cat with retrobulbar involvement and one with mandibular involvement attained a partial remission. Radiation doses ranged from 8 to 40 Gy. Four cats received concurrent chemotherapy. The median remission time for the cats that attained complete remission was 114 weeks, and three cats were alive and disease free at 131 weeks. Three cats developed disease outside the radiation field, which suggests that adjuvant chemotherapy or extending the field for part of the treatment to include additional nodes may improve loco-regional control.

In a different series, 10 cats with nasal lymphoma received chemotherapy and either 51 Gy in 3 Gy fractions or 30 Gy in 10 Gy fractions.[77] The cats that received 51 Gy had a response rate of 75%, compared with a 66% response rate in the cats that received 30 Gy. The cats that responded to treatment were all alive and disease free at intervals ranging from 4 to 55 months.

Radiation has also been used to treat extranodal lymphoma in dogs. Radiotherapy of localized cutaneous lymphoma has been reported to result in prolonged remission times. Humans with cutaneous B-cell lymphoma commonly undergo radiation therapy, which generally results in long-term control of disease. The extent of skin involvement and the presence of extracutaneous disease are prognostic.[73]

Mycosis fungoides is treated with total skin electron irradiation in humans. As with cutaneous B-cell lymphoma, the stage of disease is prognostic, but patients with disease confined to the skin may have prolonged remission times.

Interest has developed in the use of half-body radiotherapy to treat canine lymphoma. A dosage of 8 Gy to lymphoma cells reduces the surviving fraction to 0.005, which is much greater than the estimated cell kill from one cycle of chemotherapy.[68] The rationale for adding radiation to a chemotherapy protocol is that the projected improvement in cell kill would improve the duration of remission and perhaps result in a cure. Essentially, the radiation is used as an extra cycle of chemotherapy in the hope that resistance associated with chemotherapy does not affect radiation-associated cell death. However, because of the impact of radiation on normal bone marrow cells, the radiation can be administered only to one half of the body at a time. (A dosage of 8 Gy delivered to the entire body at one time would result in lethality.) In one study, induction chemotherapy (11 weeks) was administered to 94 dogs, 52 of which received subsequent half-body irradiation. Half-body irradiation was administered in 2 daily fractions of 4 Gy to the cranial half of the body; 1 month later, the same protocol was used for the caudal half of the body. No additional chemotherapy was administered, because the main purpose of the study was to see whether the half-body irradiation could serve as a substitute for long-term chemotherapy. The median survival of 311 days was comparable to but no better than many chemotherapy-only protocols.

In another study, half-body irradiation was interposed within a 25-week protocol based on cyclophosphamide, doxorubicin, Oncovin, and prednisone (CHOP). The radiation was well tolerated, and the median remission and survival times were 455 and 560 days, respectively. Although these results are very encouraging, the study involved only eight dogs, and the role of radiation in the treatment of systemic lymphoma requires further evaluation. Nevertheless, the addition of irradiation represents a new approach to a disease for which the duration of remission with chemotherapy alone has not improved over the past 10 years. Interestingly, the two half-body studies described previously differed from earlier reports in which each half-body treatment was delivered in a single 7 to 8 Gy fraction.[78] Dividing the dose into 4 Gy fractions delivered on consecutive days reduced the number and severity of acute effects. Diarrhea, the most common effect, was seen in about two thirds of the dogs after the caudal half of the body was treated.[79]

PALLIATIVE RADIATION THERAPY

Palliative radiation therapy is commonly used in human medicine, and its use in veterinary medicine has increased in recent years. The goal of palliative radiation therapy is not to provide long-term or definitive tumor

control; rather, it is intended to relieve pain or improve function or quality of life in patients in which other factors (e.g., advanced metastatic disease) are likely to lead to early demise. Palliative radiation therapy has been used most often for metastatic or primary bone tumors, principally canine osteosarcoma (see Chapter 23). Palliative therapy is relatively uncomplicated for the owner, and the cost is modest compared with curative radiation therapy because only a few fractions are administered. Curative-intent radiation protocols require strict adherence to radiation biologic principles; palliative radiation protocols, on the other hand, are far more flexible. As in human hospital settings, the protocols may vary dramatically from radiation center to radiation center.

The mechanism of the amelioration of pain caused by bony neoplasia is not completely understood. Relief of pain may occur almost immediately or may be delayed, sometimes as long as 2 weeks. Human studies have indicated that single, coarsely fractionated radiation may be comparable or superior to multifraction protocols using more conventional doses per fraction.[80] In veterinary medicine, 7 to 10 Gy fractions have been administered on days 0, 7, and 21.[81,82] In one study, 12 of 15 dogs with appendicular bone tumors treated with palliative radiation therapy had improved limb function, and the median survival time was 130 days.[81] In another study, dogs with appendicular osteosarcoma were given either 3 fractions of 10 Gy or 2 fractions of 8 Gy.[83] Seventy of the 95 dogs experienced pain relief, with a median duration of 73 days. No difference in response was found between the two treatment groups. For clients who must travel a significant distance, weekly treatments can be inconvenient. Giving 2 fractions of 8 Gy on sequential days or 1 10-Gy fraction may produce comparable palliation.[84]

Sometimes localized pain recurs before metastatic disease becomes life limiting. Palliative radiation can be readministered as long as the owners understand that continued administration of large doses per fraction eventually leads to late effects. Palliation also is useful for other tumor types that may be causing airway, bowel, or urinary tract obstruction or neurologic dysfunction. Mediastinal lymphoma often responds rapidly to irradiation. Relief from respiratory distress can be achieved within hours of a single dose of radiation.

NORMAL TISSUE TOLERANCE

Reactions from radiation therapy are classified as early or late. Late effects involve more slowly proliferating tissues, such as bone, lung, heart, kidneys, and spinal cord. When late effects occur, they may be quite severe and can result in severe fibrosis, necrosis, loss of function, or even death. The dose of radiation administered is limited by the tolerance of the normal tissue structures in the field. Late reactions can be difficult to treat. Severe late reactions should be treated under the guidance of or referred to a surgeon experienced in dealing with radiation injury.

Early effects occur during or shortly after radiation therapy. Early effects involve rapidly proliferating tissues, such as the oral mucosa, intestinal epithelium, and epithelial structures of the eyes and skin. These effects generally are self-limiting, and recovery is rapid. However, early effects can be unpleasant for the patient and distressing to the owner, and in rare instances they can result in the demise of the patient if the proper care is not given. The referring veterinarian often is called upon to treat recently irradiated patients. Treatment is based on common sense, supportive care, and the knowledge that the signs will resolve within a few weeks or occasionally months.

Mucositis

Mucositis of the oral cavity, pharynx, and/or esophagus can occur when tumors of the head and neck region are irradiated. It always occurs to some degree in patients that have received irradiation for oral tumors. The mucositis begins to develop during the second week of therapy and reaches a maximum severity during the last week of therapy. Clinical signs include thickened saliva and tenderness of the mouth. These patients may become reluctant to eat or drink, and if they are not treated, they can become dehydrated, malnourished, and debilitated. Low-salt foods are more palatable and less irritating to the oral mucosa than regular commercial diets. Hand feeding and pampering by the owner help maintain caloric intake. The owner should be instructed on the specific caloric and fluid requirements of the animal and assisted in developing a diet that meets those needs. Administration of fluids, usually subcutaneously, may be necessary. In some animals, placement of a gastrostomy or esophagostomy tube may be necessary to facilitate feeding. *Oral mucositis should subside 2 to 3 weeks after therapy.* Mucositis also can occur whenever any portion of the alimentary system receives radiation therapy. Colitis is a common acute effect during radiation therapy for bladder or colorectal tumors. Severe large bowel diarrhea may be seen. Anusitis from irradiation is worsened by the diarrhea, making the patient quite uncomfortable. High-bulk diets are recommended, along with good hygiene in the region. Steroid enemas seem beneficial in some patients with colitis.*

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*Proctofoam HC; Schwarz Pharma, Inc.; Milwaukee, Wisconsin.

Skin

Early effects to the skin are restricted to the radiation field. The severity of effect is dose related, and the patient may have a variety of lesions. Epilation is common and in some cases may be permanent. The hair may not return for several months, and the amount of regrowth varies in relation to the dose administered to the skin and the individual patient's sensitivity. Damage to the melanocytes may result in hypopigmentation or hyperpigmentation of the skin and/or alteration of the coat color when regrowth occurs. Dry desquamation may accompany epilation; this generally does not cause any problem or discomfort for the patient and usually is not treated. Moist desquamation, which usually appears 3 to 5 weeks after the start of therapy, is associated with pruritus, which can vary in severity. Self-inflicted mutilation exacerbates the problem. If moist desquamation develops, the area should be gently cleansed with warm water. Petroleum-based products should not be applied to the wound. Drying agents can be used, but no evidence of their efficacy exists. Preventing self-mutilation and good general hygiene are of paramount importance. Elizabethan collars, side braces, and/or padded bandages on the paws may be indicated, depending on the location of the field. *Moist desquamation should subside 2 to 4 weeks after it first appears.*

Eyes

Effects to the eyes are dose related and vary in severity.[85] The eye is avoided if at all possible in radiation therapy, but sometimes the location of the tumor makes it impossible to exclude the eye from the radiation field. Acute effects include blepharitis, blepharospasm, conjunctivitis, and the development of keratoconjunctivitis sicca (KCS). KCS is treated with artificial tears and steroids to prevent corneal ulceration. If corneal ulceration is present, healing may be delayed as a result of radiation damage to the corneal stem cells. KCS may be temporary or permanent, depending on the dose administered and the sensitivity of the patient. Late effects include vascular changes, which may have subtle effects on vision but in most cases do not result in blindness. Radiation-induced cataracts may occur but take years to develop fully. These cataracts can be removed with phacoemulsification. Eyes that are in the field of irradiation may receive the full treatment dose. At doses above 40 Gy, degenerative angiopathy of retinal vessels can progress over 2 years and result in retinal degeneration. Optic nerve axonal degeneration has occurred secondary to the retinal changes.[86] Newer delivery techniques, such as tomotherapy (see Figure 12-1) and IMRT, will allow superior conformal avoidance of the eye and may prevent many if not all of these problems.

BASIC PRINCIPLES OF RADIATION ONCOLOGY

Ionizing radiation kills cells by the discrete deposition of energy on or near deoxyribonucleic acid (DNA), which eventually leads to cell death. Proliferating cells are by definition radiation sensitive. Proliferating cells include both tumors and renewing cell populations (e.g., epithelial stem cells). The effects of radiation on normal, rapidly proliferating tissue systems occur during the course or shortly after radiation therapy. These types of tissues are referred to as *acutely responding* or *early responding* normal tissues. More slowly dividing and nondividing cells (e.g., bone and cells of the nervous system) are also affected by radiation, although the changes may take longer to become apparent. These types of tissues are referred to as *late-responding* normal tissues. A certain amount of damage to acutely responding normal tissues occurs in every radiation therapy patient. The total dose of radiation for most patients depends on the probability of damage to the late-responding tissues. The goal of radiation therapy is to destroy the reproductive capacity of the tumor without excessive damage to surrounding normal tissues. This goal is best achieved by dividing the *total dose* into a number of smaller fractions *(fractionation)* that are administered over a period of *time*. The relationship of these three parameters must be carefully considered in the development of radiation treatment plans, because the time-dose-fractionation relationship is critical for successful radiation therapy.

Fractionation

Early radiation oncologists found that higher total doses could be given if the doses were divided into smaller fractions. They observed that tumor response was improved, and less injury of normal tissue occurred. The response of tumor and normal tissues in the intertreatment interval between fractions, although still not well understood, has been described by Withers[63] as the four *R*s of radiation therapy: *repair* of DNA damage, *redistribution* of cells in the cell cycle, *reoxygenation* of tumor cells, and *repopulation* of tumor and normal tissues. All these processes occur between dose fractions throughout the course of radiation therapy.

Division of the total radiation dose into fractions is important for a number of reasons. The first reason is to exploit potential differences in *repair* capabilities between tumors and normal tissues. Mammalian cells

have been shown to repair a portion of the damage caused by irradiation. This repair proceeds rapidly in most tissues and is nearly complete within 24 hours after administration of a fraction. Early- and late-responding tissues differ in their response to radiation. Although slowly dividing cells appear to be somewhat less sensitive to small doses of radiation than more rapidly dividing cells, they appear to become relatively more sensitive if radiation is delivered in larger doses per fraction. If smaller doses per fraction are used, late-responding tissues can be spared relative to tumor and acutely responding tissues.

Another event that occurs between radiation fractions is cell redistribution. Tumors and replicating normal tissues proliferate by mitosis. After an interval has elapsed, cells divide again. The interval from mitosis to the next mitosis is known as the *cell cycle time*. Between mitotic events, the DNA must divide to undergo subsequent mitosis. The period of the cell cycle in which DNA undergoes synthesis is known as the *S-phase*. Before and after the S-phase are periods without overt activity by the DNA; these periods are called the G_1 *phase* and the G_2 *phase*. Cells are distributed throughout the cell cycle. The sensitivity to irradiation varies, depending on which phase of the cell cycle the cells are in at the time of irradiation. Cells in late S-phase are most resistant to irradiation, and cells in mitosis are most sensitive. When a fraction of radiation therapy is administered, many of the cells in the sensitive portions of the cell cycle are killed. During the interval between fractions, cells from the late S-phase, which are more likely to be alive than other cells, progress to more sensitive parts of the cell cycle. This is known as *redistribution*.

Because of their rapid growth and abnormal vasculature, tumors often become partially hypoxic. This is an important factor in the response to irradiation, because lack of oxygen results in less radiation damage to the DNA. Fortunately, during the interval between radiation fractions, many of the hypoxic tumor cells become aerobic and thus more sensitive to irradiation. This is known as tumor *reoxygenation*.

Many methods of exploiting the four *R*s have been studied, such as (1) using smaller doses per fraction to capitalize on the ability of late-responding tissue to repair; (2) balancing the overall treatment duration to overcome tumor proliferation during radiation therapy without causing excessive injury; and (3) using hypoxic cell sensitizers.

Time

The length of time over which radiation therapy is administered is important primarily because of tumor *repopulation* but also because of rapidly proliferating normal tissues, such as mucosa and skin. Tumor cells

that have not been destroyed by irradiation continue to replicate during the course of therapy. This process is exacerbated by a phenomenon known as *accelerated repopulation*. Some have suggested that after approximately 4 weeks of therapy, tumors repopulate more rapidly than initially.[64] The reason for this is not clear, but the phenomenon could be related to (1) a reduction in the cell cycle time, (2) an increase in the number of tumor cells that are actively dividing, or (3) a reduction in the number of tumor cells that normally die (cell loss factor). Regardless of the cause, when treatment lasts longer than 4 weeks, repopulation *may* affect the outcome. Accelerated repopulation may have a greater impact on rapidly dividing tumors than on slowly dividing tumors.

Acutely responding normal tissues are also affected by time. These tissues also repopulate during radiation therapy. The same total dose of radiation administered over a short period results in somewhat more severe acute effects than if administered over a longer course. However, although acute effects may cause discomfort for the patient, the effects generally are self-limiting and can be managed with supportive care.

Late-responding normal tissues are not significantly affected by the length of time over which therapy is administered. Fraction size is a far more important consideration. The time *between* treatment fractions is important. Some treatment protocols advocate multiple fractions per day to administer the total dose in a short period to prevent repopulation, but in smaller fraction sizes to minimize damage to late-responding tissues. Fractions should be separated by at least 6 hours to allow repair of DNA damage to normal tissues. Cells of the brain and spinal cord may require additional time for complete repair, and the impact of multiple fractions per day on these late-responding tissues is not clearly understood.

Total Dose

The radiation dose is delivered in units known as *Gray*. A Gy is 1 Joule/kg and is equal to 100 rad, the previously used measure of radiation dose. The total dose administered to a patient should have a low probability for causing significant late normal tissue reactions in the region of therapy. However, as mentioned previously, the response of these tissues depends on the fraction size. For example, 48 Gy administered in 4 Gy fractions has a much higher probability of causing late effects than 48 Gy administered in 3 Gy fractions (Figure 12-2). The probability of tumor control is thought to be fairly similar, because acutely responding tissues, like tumors, are not as sensitive to the fraction size by which the dose is administered. The benefits of protocols that use small doses per fraction are clear: they allow a higher total dose to be administered without increasing the probability of
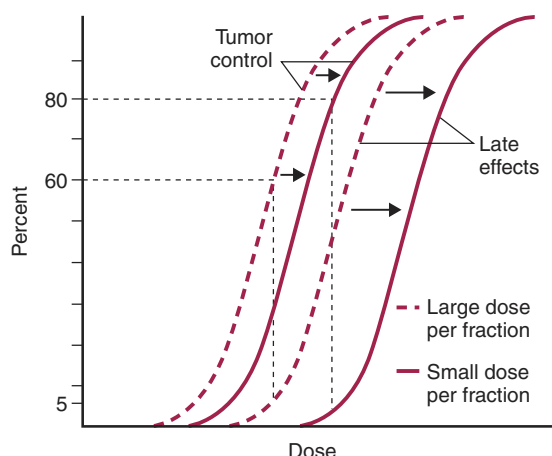
Figure 12-2

Radiotherapy delivered in small fractions *(solid lines)* can produce a higher probability of tumor control with the same level of late effects as radiotherapy delivered in large fractions *(broken lines)*.

damage to late-responding normal tissues. This higher dose translates into a better probability of tumor control. However, gains from this approach can be lost if the radiation protocol is spread over a length of time that allows tumor repopulation to begin adversely affecting tumor control. The total dose tolerated is also dependent on the type of late-responding normal tissue in the irradiated field. For example, brain and spinal cord are less tolerant to the effects of irradiation than muscle or bone. Another factor that must be considered when selecting the appropriate dose is the volume of tissue in the field. Large volumes of normal tissues are more susceptible to damage from irradiation than smaller volumes.

No perfect radiation therapy protocol exists, and all protocols commonly used in veterinary and human medicine have advantages and disadvantages. It is not within the scope of this chapter to prescribe specific radiation doses or fractionation schedules, because many factors must be considered. Rather, referring veterinarians must know what to expect when sending patients to a radiation oncology center, and they must be able to explain some of the fundamental principles to clients. The radiation oncologist should inform the referring veterinarian and owner of the probabilities of late effects and tumor control and estimate the degree of acute effects expected with a specific protocol.

RADIATION THERAPY EQUIPMENT

Ionizing radiation can be administered by an external source (teletherapy), through placement of radioactive isotopes interstitially (brachytherapy), or by systemic or cavitary injection of radioisotopes, such as iodine-131 (^{131}I). Teletherapy, also referred to as *external beam radiation therapy*, is the most commonly used method of radiation therapy in veterinary medicine. External beam radiation therapy usually is classified as orthovoltage or megavoltage radiotherapy, based on the energy of the photon. Orthovoltage machines produce x-rays with an energy of 150 to 500 kVp; megavoltage radiation emits photons with an average energy greater than 1 million electron volts (1 MeV). Although some veterinary radiation oncology centers continue to treat with orthovoltage machines, megavoltage radiation, which is used almost exclusively at human centers, is becoming more common. Megavoltage radiation for therapy can be obtained from cobalt machines or linear accelerators. Because megavoltage radiation has excellent tissue penetrating capabilities, radiation therapy can be performed on deeply seated tumors for which orthovoltage therapy would not be an option. Radiation therapy is still performed at some centers with cesium machines. Cesium emits photons with an energy of 662 keV, providing limited skin sparing but a more even dose distribution than orthovoltage therapy.

Orthovoltage x-rays, which have low energy, distribute maximum doses to the skin surface. Acute effects to the skin can be quite severe, causing discomfort to the patient, and late effects to the skin and subcutaneous tissues can be dose limiting. Megavoltage radiation has a higher energy than orthovoltage, and the photons must interact with tissues, allowing the dose to build up, before the maximum dose can be achieved. The skin therefore can receive a significantly weaker dose than the underlying tumor. This skin-sparing effect of megavoltage radiation allows the optimal dose to be administered to the more deeply seated tumor without causing severe reactions to the skin. When the tumor involves the skin, megavoltage radiation can be used successfully by placing a sheet of tissue-equivalent material, called a *bolus*, over the tumor. This allows the dose buildup to occur before reaching the skin, so that the skin and associated tumor can receive the maximum dose of radiation.

The absorption of megavoltage radiation, unlike that of orthovoltage radiation, is minimally dependent on the composition of the tissue. This characteristic permits even distribution of the dose throughout the tissues in the field. Orthovoltage radiation is preferentially absorbed by bone. If a tumor adjacent to or overlying bone is administered a meaningful dose of orthovoltage radiation, the probability that late effects to the bone (bone necrosis) will develop is quite high. Treatment with orthovoltage should be limited to small, superficial tumors, such as nasal planum tumors, or to superficial tumor beds after surgical excision. Most veterinary radiation therapy centers now use megavoltage radiation.
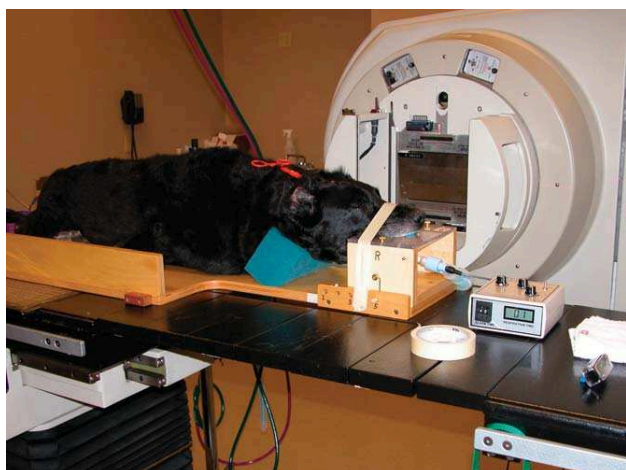
Figure 12-3

A bite plate is made before imaging to facilitate treatment planning. The patient is repositioned each day, using the plate, so that positioning is consistent. (Courtesy Pamela Jones.)

Figure 12-4

A 3DCRT treatment plan for a cat with a vaccine-associated sarcoma. The block is depicted on the plan, and a template is created so that a custom-made block *(right)* can be produced.

The interaction of megavoltage radiation with tissues is quite predictable, which has allowed the development of computerized treatment planning systems. These planning systems allow treatment of the tumor with multiple beams administered from different angles. Beam modifiers, such as wedges and blocks, can be incorporated into the treatment plan. Wedges are triangular-shaped pieces of lead that can be placed between the beam and the patient. Less radiation penetrates the thick side of the wedge, which modifies the dose distribution. The goal of computerized treatment planning is to ensure a desired minimum tumor dose to a region specified by the radiation oncologist and to spare normal tissue structures when possible. Conventional radiation therapy uses a limited number of CT or MRI images, which are imported or contoured using a tablet. The summation of multiple beams coming from different directions provides a higher dose to the tumor than surrounding tissues.

Advances in treatment planning and imaging over the past decade have led to the development of image-based, 3D conformal radiation therapy (3DCRT), which permits better conformity between the irradiated high-dose volume and the geometric shape of the tumor. 3DCRT requires importation of CT, MRI, or positron emission tomography (PET) imaging into the treatment planning system. The animal must be positioned for the imaging in a fashion that can be replicated precisely on a day-to-day basis for treatment. Alpha cradles or bite plates (Figure 12-3) often are used as positional aids. The radiation oncologist identifies important normal tissue structures and the tumor and target volumes on these images. More sophisticated shaping is performed by taking advantage of multileaf collimators
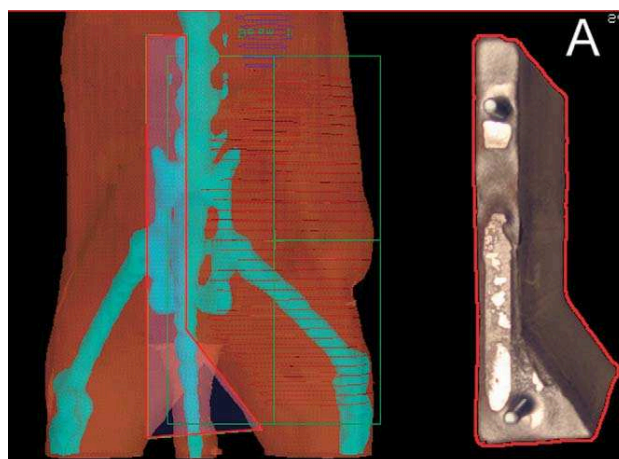
or custom-made blocks (Figure 12-4). A major advantage of 3DCRT is that dose-volume histograms can be obtained for the tumor and normal tissue structures (see Figure 12-1). This provides a quantitative method of evaluating treatment plans and enhances quality assurance. Defined dosimetric parameters may be useful predictors of outcome.

IMRT, tomotherapy, and stereotactic radiosurgery allow even greater sculpting of the radiation dose. All these modalities require strategies for patient positioning and immobilization. IMRT is an advanced form of 3DCRT in which the collimator leaves are computer driven during treatment, allowing the beam intensity to vary within each portal. More beams are used than in conventional radiation therapy, and a highly sophisticated, inverse-treatment planning algorithm is required. Fractionation schedules similar to those for conventional radiation therapy are used, but the dose to the tumor is higher, whereas the dose to surrounding tissues remains at tolerance. This provides therapeutic gain and should lead to better tumor control. Tomotherapy is a form of IMRT that uses a helical delivery system to sculpt the beam. Tomotherapy has had limited use in veterinary medicine, and outcome data have not been reported.[87] IMRT could prove useful for the treatment of large tumors with complex geometry located near important normal tissues; such tumors include nasal tumors, oral tumors and, in cats, vaccine-associated sarcomas. Radiosurgery may use technology similar to that for IMRT or may be delivered with a cyberknife.[88] The difference between radiosurgery and IMRT is the biologic approach. In radiosurgery, larger doses per fraction generally are administered in fewer treatments (one to five), making it more convenient.[89]

Stereotactic frames or image-guided systems must be used to ensure that the dose is administered precisely. Radiosurgery is ideal for small tumors or for palliative treatment of tumors that are near crucial normal tissue structures. Radiosurgery has been used on a limited basis in veterinary medicine for brain and bone tumors.[51,88]

Technologic advances such as those described previously are likely to become available on a limited basis over the next decade. An important consideration is identifying which tumor types will benefit most from such approaches.

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Immunotherapy of Cancer

Barbara J. Biller and Steven Dow

The powerful ability of the immune system to recognize and eliminate cancer, based on exquisitely subtle differences between normal and malignant cells, is the fundamental rationale for the immunotherapy of cancer. Many lines of evidence support a role for the immune system in controlling cancer. These include (1) the occurrence of spontaneous remissions in cancer patients without treatment, (2) the increased incidence of some types of cancer in immunosuppressed patients, (3) the presence of tumor-specific cytotoxic T cells within tumor tissue or tumor-draining lymph nodes, and (4) the development of paraneoplastic autoimmunity.

As the scientific tools used to study tumor immunology at the molecular level become increasingly sophisticated, immunotherapy is becoming more precise and effective. The advances made in the past decade now make it possible to specifically target therapy toward critical aspects of the tumor without damaging normal tissues or eliciting systemic toxicity. Monoclonal antibodies against vascular endothelial growth factor, for example, target tumor vasculature with little toxicity to normal tissues.[1] In contrast, many early forms of immunotherapy, such as the mixed bacterial vaccines, were relatively toxic, causing local reactions and systemic toxicity.[2,3]

The elucidation of the complex relationship between the immune system and cancer reveals the potential pitfalls of immunotherapy, as well as the possibilities. For example, therapy with IL-2, a cytokine long recognized for its importance as a T cell growth factor, has been found to support the growth and expansion of regulatory T cells. These regulatory T cells generally have a immunosuppressive function and frequently interfere with the development of effective antitumor immunity.[4] Similarly, the cytokines TGF-β and IFN-γ, both important regulators of the adaptive immune response, cause tumor inhibition in some situations but stimulate tumor growth in others.[5,6]

Despite the challenges, effective tumor immunotherapy is becoming a reality. This represents a significant step toward the development of a new class of therapeutics with a more precise and targeted approach to the treatment of cancer. In the not too distant future, it is likely that immunotherapy will have a place alongside surgery, radiation therapy, and chemotherapy in the routine treatment of many malignancies. In this chapter we discuss the major types of immunotherapy being evaluated in clinical trials or already used in practice, focusing on the scientific basis for their use as well as their limitations in both physician- and veterinary-based oncology.

TUMOR IMMUNOLOGY

Cellular Components of the Antitumor Immune Response

The immune response as a whole can be divided into two main components: the rapidly acting innate immune response and the more specific but more slowly developing adaptive immune response. Innate immunity includes physical and chemical barriers to pathogens, such as skin and mucosal epithelia, the complement system, and phagocytic cells such as macrophages, dendritic cells (DCs), neutrophils, and natural killer (NK) cells. Adaptive or acquired immunity consists of B and T lymphocytes; the T cells are further subdivided into cytotoxic lymphocytes that express the CD8 co-receptor and bind major histocompatibility (MHC) class I molecules and helper T cells that express the CD4 co-receptor and recognize MHC class II molecules. The cells of both innate and adaptive immunity, especially DCs, macrophages, and T lymphocytes, produce or respond to a variety of cytokines and chemokines that shape the nature of the overall immune response. Cytokines with particular relevance to cancer immunotherapy are listed in Box 13-1.

Innate immunity
Dendritic Cells. The innate immune response is driven by the professional antigen presenting cells (APCs), primarily dendritic cells, and macrophages. DCs are the most potent of the APCs and initiate an antitumor

Box 13-1	Biological Activities of Key Cytokines Relevant to Tumor Immunotherapy

Cytokine	Major activity
IL-2	Growth factor for T cells, including regulatory T cells; induces proliferation and differentiation of T cells to effector T cells, enhances CTL and NK cell cytotoxicity and production of LAK cells, induces B-cell proliferation
IL-3	Multicolony-stimulating factor; promotes production/differentiation of macrophages, neutrophils, eosinophils, mast cells, and basophils and supports growth of pluripotent progenitor cells
IL-4	Key Th2 cytokine; induces differentiation of naïve CD4+ T cells toward Th2 phenotype, inhibits macrophage activation, induces B cell growth and differentiation, stimulates isotype switching and IgG and IgE production
IL-6	Supports B cell proliferation and differentiation to plasma cells; pro-inflammatory, antiapoptotic cytokine that may contribute to tumor development associated with chronic inflammation
IL-8	Chemotactic/activation factor for neutrophils and T cells; induces matrix metalloproteinase-2 activity, plays a role in inflammation and tumor metastasis
IL-10	Immunosuppressive cytokine produced by activated DCs, macrophages, and T cells; induces regulatory T cell function; also overexpressed by some tumors and tumor-associated leukocytes
IL-11	Stimulates proliferation of hematopoietic stem cells, induces megakaryocyte maturation resulting in increased platelet production
IL-12	Key Th1 cytokine produced by DCs and macrophages, stimulates synthesis of IFN-γ by T cells and NKs, enhances cytotoxicity of CTLs and NK cells, stimulates differentiation of naïve CD4+ T cells to T cells with the Th1 phenotype
IL-13	Th2 promoting cytokine, produced by NKT cells; inhibits inflammatory cytokine production by macrophages, possible inhibitory role in tumor immunosurveillance
IL-15	T cell growth factor; supports survival of memory CD8+ T cells, promotes NK cell activation and survival and triggers cytotoxic activity
IL-19	Promotes T cell differentiation toward the Th2 phenotype
IL-21	Member of IL-2 cytokine family; enhances cytotoxicity of CTL and NK cells
IL-23	Member of IL-12 cytokine family; produced by activated DCs, stimulates Th1 cell-mediated immune response
GM-CSF	Promotes growth and differentiation of pluripotent progenitor cells, stimulates growth of cells of the granulocyte, macrophage, and eosinophil lineage
IFN-α,β	Induce apoptosis of tumor cells, enhances CTL effector function, activates NK cells, modulates MHC class I/II expression, inhibits tumor angiogenesis
IFN-γ	Key Th1 cytokine produced by activated T cells and NK cells; promotes the differentiation of naïve CD4+ T cells to Th1 phenotype, activates macrophages, increases MHC class I/II expression
TNF-α	Produced by Th1 T cells, CTLs, activated DCs, and macrophages; induces NO production by macrophages, induces tumor apoptosis; important proinflammatory cytokine
TGF-β	Immunosuppressive cytokine; inhibits macrophage activation and B cell growth; overexpressed by some tumors

immune response through phagocytosis and processing of tumor-derived antigens. To properly activate tumor-specific T cells, DCs must be activated by a "danger signal," such as heat shock protein or IFN-α, that enables recognition of transformed or damaged cells.[7] This leads to expression of costimulatory molecules, such as CD80 and CD40, on the mature DC surface, DC migration to a tumor-draining lymph node, and antigen presentation and activation of T lymphocytes.

DCs are generally thought of as helpful in terms of tumor control since they initiate the immune response, but in some circumstances DCs are detrimental to the development of an effective antitumor response. Munn et al. demonstrated that plasmacytoid DCs (a specific DC subset) within tumor draining lymph nodes can potently suppress antitumor T cells.[8] This inhibitory effect arises from constitutive expression of indolemine-2,3-dioxygenase (IDO), an enzyme that metabolizes the breakdown of tryptophan, which is an essential amino acid required for normal T cell function.[9]

Macrophages. Macrophages are very efficient phagocytic cells that recognize invading pathogens as well as damaged, apoptotic, or malignant cells. Upon phagocytosis, macrophages produce toxic products such as nitric oxide (NO) and hydrogen peroxide that have bactericidal and tumoricidal properties.[10,11] Activated macrophages release key cytokines, primarily TNF-α, IL-1, IL-6, and IL-12, which induce both a local inflammatory response and systemic immune response through the subsequent activation of neutrophils, DCs, NK cells, and T cells. Macrophages also kill tumor cells through antibody-dependent cellular cytotoxicity (ADCC). This was demonstrated in canine pulmonary alveolar macrophages where activated macrophages mediated potent *in vitro* cytotoxicity against monoclonal antibody-labeled melanoma cells.[12] The macrophage-mediated cytotoxicity was significantly greater than the tumor cell killing induced with the monoclonal antibody alone.

Macrophages, like DCs, can also promote tumor growth and progression. Depending on the cytokines and chemokines produced by the tumor and stroma, macrophages are polarized into one of two types: M1 or M2. M1 macrophages activate cell-mediated immunity through production of IL-12, IFN-γ, and TNF-α and are potent producers of NO. In contrast, M2 macrophages produce immunosuppressive cytokines, such as IL-10 and TGF-β, and are poor producers of NO and other tumoricidal products.[13]

Tumor-associated macrophages are generally polarized to the M2 phenotype and thus may contribute to tumor evasion of the adaptive immune response.

Natural Killer Cells. Natural killer (NK) cells play an important role in the early immune response to tumors. Similar to cytotoxic CD8+ T lymphocytes (CTLs),

NK cells mediate tumor cell cytotoxicity through the release of perforin granules that induce apoptosis. NK cells, however, recognize tumor cells in a completely different manner than CTLs and are thought to participate in the early recognition of transformed cells.[14,15] This occurs in two different manners. The first is through detection of abnormally low levels of MHC class I expression, a common alteration on the surface of tumor cells. When an NK cell encounters a cell lacking appropriate levels of MHC class I (the "missing self" hypothesis), normal inhibitory signals are lost and the activated NK cell mediates tumor cell killing.[16]

The second mechanism of recognition is mediated through a family of molecules known as the MHC class I chain-related (MIC) proteins. Two ligands, MICA and MICB, have been identified on the surface of cells exposed to stressful stimuli such as radiation damage, oxidative stress, or chronic inflammation. Binding of damaged cells expressing MICA or MICB leads to NK cell activation and apoptosis of the transformed cell.[17,18]

Adaptive immunity

CD8+ T Cells. Traditionally, much of the focus of tumor immunology has been directed toward understanding the role of tumor-specific cytotoxic T lymphocytes. Following antigen presentation by dendritic cells, in the context of MHC class I and costimulatory molecules, antigen-specific CD8+ T cells differentiate into CTLs. Tumor-specific CTLs can lyse tumor cells directly, through release of perforin granules, or indirectly, through release of cytokines that further boost the cell-mediated immune response. But to be clinically effective, tumor-specific CTLs must also be able to recognize tumor antigens with high avidity as well as infiltrate the tumor stroma. In mice transgenic for T cell receptors that recognize tumor antigens, nearly all of their CTLs recognize tumor, but tumor growth is unaffected.[19] Although many cancer vaccines elicit robust CTL responses, objective tumor regression occurs much less frequently.[20]

CD4+ T Cells. Another reason for the discrepancy between a demonstrable immunological response and tumor regression lies in the importance of CD4+ T cell help in priming and maintaining antigen-specific CD8+ T cells. Following initial priming, CD4+ T cells differentiate into either T-helper 1 (Th1) cells, which produce IFN-γ and support CD8+ CTL responses, or T-helper (Th2) cells, which produce IL-4, IL-5, and IL-13 and support humoral (B cell) immunity. CTLs that do not receive supporting signals from Th1 T cells may not be sufficiently activated or do not survive long enough to mediate effective tumor cell killing. CD4+ T cells are also essential for the generation of long-lived, functionally active CD8+ memory T cells which are required for the

development of long-lasting immunity and protection from relapsing disease.[21,22]

B Lymphocytes. The effector functions of antibodies, which are produced by B cells, include activation of the complement system, enhanced phagocytosis of opsonized target cells and induction of ADCC. While somewhat controversial, B cell responses to tumors are thought by many to be less important than the development of T cell–mediated immunity. Although a few studies correlate improved prognosis with induction of antitumor antibodies, many cancer patients have malignant tumors in the presence of high titers of antibodies.[23] The most important aspect of antibody-mediated immunotherapy is the development of monoclonal antibodies that target well-defined tumor antigens or overexpressed self antigens. This topic is covered in more detail in Section IV.

Immune Surveillance of Cancer

The concept that the immune system can actively prevent the development of neoplasia in the host is termed "cancer immunosurveillance." This theory, put forth more than 40 years ago by Thomas and Burnet, was initially based on the finding that lymphocytes can recognize antigens on newly transformed cells and eliminate them before a clinically evident tumor develops.[24,25] When this concept was put to the test several years later in immunodeficient mice, increased incidence of either carcinogen-induced tumors or spontaneous cancer development was not detected.[26,27] Although we know now that this was the result of residual immunity in these mice, the cancer immunosurveillance concept fell from favor and for decades was viewed as being unfounded and incorrect.

Renewed interest in immunosurveillance began about the early 1990s, coincident with significant improvement in the tools and techniques necessary to evaluate specific components of the immune response. Sound scientific evidence now supports some aspects of the cancer immunosurveillance hypothesis. For example, Kaplan et al. found that the cytokine IFN-γ, an important product of cytotoxic T cells, could protect mice against the growth of transplanted or chemically induced tumors. Mice lacking the receptor for IFN-γ were more sensitive to methylcholanthrene (MCA) induced sarcomas than normal mice.[28] Furthermore, IFN-γ receptor (IFN-γR) deficient mice were more likely to spontaneously develop sarcomas and lymphomas following mutation of the p53 tumor suppressor gene than mice with p53 mutations and normal IFN-γ receptor function.[28,29]

Some of the most compelling evidence for cancer immunosurveillance stems from studies in mice that lack the recombination-activating gene (RAG). These mice lack major components of the adaptive immune response, including T and B lymphocytes.[30] RAG deficient mice have a high rate of spontaneous gastrointestinal and mammary neoplasia as well as chemically induced sarcomas. Combination of the RAG mutation with defective IFN-γ production results in spontaneous cancer formation in young mice, often before 18 months of age.[31,32]

Despite these data, the tumor immunosurveillance theory remains controversial. Recently Rodrigues and Travassos showed that IFN-γR deficient mice were resistant, rather than susceptible, to tumor challenge with high doses of B16 melanoma cells. They further demonstrated that tumor resistance was mediated by the cytotoxic effects of IFN-γ accumulation within immune effector cells of the receptor deficient mice.[33] Efforts to understand the seemingly opposing roles of the immune system in shaping tumor progression or regression are a significant part of ongoing research in the tumor immunology field.

Mechanisms of Immune Evasion by Tumors

Despite the evidence for recognition and destruction of tumors by the immune system, there are significant barriers to the generation of effective antitumor immunity. The fact that tumors can evade surveillance mechanisms and proliferate in an immune-competent host is illustrated by the large numbers of both people and animals that succumb to cancer each year. There are multiple ways in which tumors evade the immune response, all of which present challenges to the successful implementation of immunotherapy of cancer.

Impaired DC activation and function

One important manner in which tumor tolerance develops is a consequence of tumor growth in the absence of dendritic cell activation.[34] Initially, most tumors do not appear dangerous to the immune system because they are closely related to self-tissues. Although tumor antigens are expressed at this stage and immature DCs take them up by phagocytosis, most antigens are weakly immunogenic and fail to fully activate the DCs. This occurs secondary to lack of proper costimulatory molecule expression on the DC surface and greatly impairs antigen presentation to T cells. T cells that encounter weakly immunogenic antigens in the absence of appropriately activated DCs are themselves inactivated (termed "anergy") and may be deleted from the T cell repertoire, resulting in permanent loss of the immune system's potential to respond to a particular tumor-derived antigen.[35]

This situation is further compounded by the finding that DCs from cancer patients are frequently abnormal and display characteristics of immature myeloid cells.

DCs from the systemic circulation, as well as intratumor DCs, express abnormally low levels of MHC and costimulatory molecules on their cell surface. Not surprisingly, these DCs are poor antigen presenting cells and tend to be immunosuppressive rather than immunostimulatory.[36] Tumor-driven production of the cytokines IL-10 and IL-6, as well as vascular endothelial growth factor (VEGF) and granulocyte-macrophage colony stimulating factor (GM-CSF), are thought to be responsible for most of the defects in DC maturation in people with cancer.

Production of immunosuppressive cytokines

Another means of immune evasion occurs through tumor production of anti-inflammatory cytokines, such as TGF-β and IL-10.[35] These cytokines potently suppress antitumor responses by acting directly on CD4+ and CD8+ T cells and through inhibition of DC function. Increased serum IL-10 is a frequent finding in people with cancer.[37,38] IL-10 expression by tumor cells within metastatic lesions and draining lymph nodes has been well-documented; Botella-Estrada found that increased IL-10 in sentinel lymph nodes was predictive of eventual metastasis in people with melanoma.[39] Tumor-induced immunosuppression likely exists in veterinary cancer patients as well. Catchpole et al. found that IL-10 and TGF-β were overexpressed within the lymph node of a dog with metastatic melanoma.[40] Interestingly, no T cell cytokines, such as IL-2, IL-4 or IFN-γ, were detected in the lymph node, suggesting tumor-induced development of an immunosuppressive environment.

Induction of regulatory T cells

A third mechanism of evasion is mediated through induction of regulatory T cells (Treg). These T cells, initially termed suppressor T cells, are a distinct CD4+ T cell subset defined in mice and humans by expression of the CD25 surface molecule, along with surface expression of CTLA-4, GITR, Lag3, and intracellular expression of the Fox P3 transcription factor.[41,42] Treg directly suppress tumor-specific CD4+ and CD8+ T cells and are attracted to the tumor in some cases by tumor expression of the chemokine CCL21.[43] Increased numbers of Treg have been documented in mice and humans with cancer. Numerous studies in people demonstrate increased Treg in patients with breast, lung, and pancreatic carcinoma; in several studies, this increased Treg expression is correlated with poor prognosis.[44–48] Additionally, Tregs are overexpressed in metastatic lymph nodes and can comprise a major portion of tumor-infiltrating lymphocytes.[49]

Preliminary work in our lab supports the existence of the Treg subset in dogs, with higher numbers of Treg detected in cancer-bearing dogs compared to healthy dogs.[50] Despite induction of clinical remission, dogs with lymphoma demonstrated increasing Treg numbers during chemotherapy with a CHOP (see Chapter 31 for CHOP definition)-based protocol. Whether this correlates with decreased remission and survival has not yet been determined.

NONSPECIFIC TUMOR IMMUNOTHERAPY

The finding that certain infectious disease processes could positively impact the treatment of malignancy is recorded in history as far back as the 18th century.[51] In the early 1900s William Coley, a New York surgeon, was instrumental in early efforts to understand this link; he noted that some cancer patients developing incidental bacterial infections survived longer than those without infection.[52] In support of his observations, studies during this time pointed to a lower frequency of cancer in patients with tuberculosis.[53] Thus, in an effort to stimulate the immune system, Coley developed a bacterial "vaccine" consisting of killed cultures of *Streptococcus pyogenes* and *Serratia marcescens* ("Coley's toxins") that he used to treat people with inoperable sarcomas. Despite remarkable success in about 15% of patients, including complete and durable tumor remission, the high failure rate and occurrence of significant side effects lead to discontinuation of this approach.[52] Coley's work, however, laid the foundation for nonspecific modulation of the immune response in the treatment of cancer.

Biologic Response Modifiers

BCG and *Corynebacterium Parvum*. The bacillus of Calmette and Guerin (BCG), a modified strain of *Mycobacterium bovis* initially developed as a vaccine for tuberculosis in the early 20th century, is an extension of Coley's work that is still in use today. Infusion of BCG into the bladder is one of the most successful forms of treatment for superficial bladder cancer in humans. In this setting, BCG is more effective than chemotherapy, especially in treating and preventing relapse of noninvasive transitional cell carcinoma.[54,55] While the precise mechanism for its antitumor effect is not clear, BCG must directly contact malignant cells, which elicits production of inflammatory cytokines such as IFN-γ, IFN-α, and IL-2. This response attracts and activates neutrophils, macrophages, and T lymphocytes, triggering cell-mediated cytotoxicity of tumor cells and induction of long-lived memory T cells that provide protection from relapsing disease.[56]

Although BCG can be instilled into the canine bladder and is well tolerated at low doses, it is not effective

in the treatment of canine transitional cell carcinoma (TCC), which generally does not present as superficial disease as it does in humans.[57] Knapp, in a review of 102 dogs with TCC, found that 98% of dogs had tumors invading the bladder wall +/− adjacent organs at the time of diagnosis.[58] More recently, Knapp et al. investigated the use of a related compound known as mycobacterial cell wall-DNA complex (MCC).[59] MCC is a bifunctional anticancer agent that induces tumor-cell apoptosis and stimulates inflammatory cytokine production in a manner similar to BCG. *In vitro*, MCC inhibited proliferation and induced apoptosis of canine TCC cells; paradoxically, induction of apoptosis was enhanced by the addition of piroxicam.

A similar bacterial product, derived from *Corynebacterium parvum*, demonstrated antitumor activity in people and dogs with melanoma when used in combination with surgery.[60,61] In dogs with advanced disease (stage II and III oral melanoma), survival was increased in dogs treated with *C. parvum* and surgery, compared to dogs treated with surgery alone.[61] Another study evaluating the effect of simultaneous intralesional administration of *C. parvum* and BCG in dogs with mammary tumors demonstrated increased survival.[62]

Attenuated Salmonella strains

Facultative anaerobic bacteria are attractive cancer therapeutics because of their selective growth in hypoxic regions of solid tumors. Genetically modified bacteria from several strains of Salmonella, including *S. typhimurium* and *S. choleraesuius*, can be specifically targeted to tumors following systemic administration. The bacteria then replicate only within tumor tissue, allowing efficient delivery of genes and other proteins to tumor tissue.

Besides their utility for targeted drug delivery, attenuated Salmonella directly inhibit tumor growth. In a mouse melanoma model, an attenuated *Salmonella typhimurium* (VNP20009) selectively targeted both primary tumors and metastatic lesions, significantly slowing tumor growth.[63] Antitumor effects are thought to occur by several different mechanisms, including production of inflammatory cytokines, such as TNF-α, and toxic proteins that induce tumor apoptosis. The bacteria stimulate innate immunity through production of an attenuated lipopolysaccharide (LPS), a potent stimulus for enhanced DC function.[64,65]

Thamm et al. recently completed a phase I clinical evaluation of systemic VNP2009 administration in dogs with a variety of malignant tumors.[66] In this study, 41 dogs received either weekly or biweekly intravenous infusions of the modified *Salmonella* product and were monitored for acute toxicity and tumor response. The dose-limiting toxicities were fever and vomiting, occurring at the high end of the dose interval in this dose-escalating trial. Colonization of the bacteria within tumor tissue was detected in about 40% of the dogs, with significant clinical responses (either complete remission or partial remission) occurring in 15% of patients. Overall, 37% of dogs experienced either disease stabilization or a transient response to treatment. Given the advanced stage of disease in the dogs of this study, the low incidence of toxicity, and the high overall response rate, further evaluation of VNP2009 is warranted.

Oncolytic viruses

Viruses that preferentially replicate in and lyse tumor cells are referred to as oncolytic. The replication cycle of many viruses utilizes the same cellular pathways that are frequently altered in cancer cells.[67] Because they are tumor selective, oncolytic viruses offer an attractive approach for targeted delivery of genes, drugs, and cytokines to malignant cells. They are also capable of direct tumor cell killing and further enhance antitumor immunity through stimulation of host innate and adaptive immune responses.

Some RNA viruses, such as reovirus, Newcastle disease virus, and vesicular stomatitis virus (VSV), are inherently tumor selective.[68] Viruses belonging to other families such as adenovirus, vaccinia, and herpes simplex can be genetically modified to preferentially infect malignant cells.[69–71] Modification of the adenoviral early genes 1 A (E1A) and 1 B (E1B), for example, generates adenoviruses that target very rapidly dividing cells but are unable to replicate in normally cycling cells. Herpes simplex virus (HSV) replication can be targeted to malignant cells through the design of mutants with defective viral genes encoding for thymidine kinase and ribonucleotide reductase. Host ribonucleotide reductase and thymidine kinase are expressed only in the G1 and S phases of the cell cycle (in which cells proliferate rapidly) so, to survive, an HSV mutant replicates in rapidly cycling cells.

Most clinical experience with the oncolytic viruses stems from trials with various forms of adenovirus, HSV, and reovirus. Of these, the adenovirus Onyx-015 has made the most progress.[72] In phase I and II trials for the treatment of people with head and neck squamous cell carcinoma, Onyx-015 was administered intratumorally. Pre- and posttreatment biopsies and clinical tumor responses were assessed. As a single agent, Onyx-015 was not effective, with detectable viral replication in only 20% of tumors and objective clinical response in 14% of patients. When combined with chemotherapy, however, Onyx-015 demonstrated remarkable clinical response in 63% of patients, with 27% (eight patients) demonstrating complete remission of all tumors. Onyx-015 has also been evaluated in phase I trials for advanced pancreatic, ovarian, colorectal, and prostatic cancer.[68]

Several variants of HSV-1 have been evaluated in phase I clinical trials.[73,74] Patients with recurrent malignant glioma were treated with stereotactic injection of the mutant virus. Neither serious side effects nor development of HSV encephalitis occurred, demonstrating the feasibility of this approach. A follow-up study documented intratumoral replication of HSV in high-grade gliomas with radiographic evidence of tumor response in several patients.[75]

The canine distemper virus, a member of the Morbillivirus family, has been tested *in vitro* for possible application to the treatment of dogs with lymphoma.[76] Canine distemper virus recognizes CD150, a cell membrane protein expressed on lymphoid cells and overexpressed on malignant B cells. Binding through CD150 induces apoptosis of the target cell. Using fluorescently labeled attenuated distemper virus, infection of canine lymphoid cell lines and lymphocytes isolated from canine lymphoma patients was demonstrated. A large proportion of the infected cells were killed, suggesting the possibility of using attenuated canine distemper virus to treat dogs with lymphoma.

Superantigens

Bacterial superantigens (SAgs), such as *Staphylococcus aureus* enterotoxin A or B (SEA or SEB), potently activate T cells through simultaneous cross-linking of the T cell receptor (TCR) and MHC class II molecules. The proliferating T cells secrete mainly Th1 cytokines such as IL-2, TNF-α, and IFN-γ. SAg activated T cells are strongly cytolytic and have been shown to induce tumor regression in mice[77,78]. SAgs can be specifically targeted to tumor tissues when fused with tumor-directed monoclonal antibodies or transfected directly into tumors. SAgs, delivered in conjunction with immunostimulatory cytokines such as IL-2 or GM-CSF, induce efficient tumor-cell lysis as well as a systemic antitumor immune response.[79,80]

Unfortunately the toxic effects of SAgs are much more pronounced in people than they are in rodents. In humans, SAgs can induce massive T cell cytokine release, in effect eliciting a toxic shock syndrome.[81] Genetically modified SAgs with reduced binding to human MHC class II retain much of their T cell activating potential with reduced risk of toxicity and are presently being evaluated in people with advanced cancer. In a phase I trial for people with nonsmall cell lung carcinoma the dose limiting toxicity was hypotension, occurring in 16% of patients. Stable disease occurred in 42% of patients, with some experiencing decreased tumor burdens by as much as 50%.[82]

The therapeutic potential of SAgs in veterinary oncology has been evaluated in clinical trials in dogs with oral malignant melanoma and soft tissue sarcoma.

A phase I/II trial by Dow et al. examined the clinical response and immune response of 26 dogs with oral melanoma treated by intratumor injection of lipid-complexed plasmid DNA encoding SEB and either GM-CSF or IL-2.[79] Toxicity was minimal in this study; histopathological examination of injected tumors revealed marked infiltrates of CD4+ and CD8+ T cells and macrophages. Significant clinical responses (complete or partial remission) occurred in 46% of dogs; survival times for dogs with stage III disease were significantly prolonged compared to dogs treated with surgical tumor excision only.

Efficacy of the lipid-DNA-SEA/IL2 compound was also assessed in dogs with spontaneous soft tissue sarcoma.[83] In a phase I/II trial, the superantigen compound was injected into the tumor once weekly for up to 12 injections. Surgery was performed post-therapy. The overall response rate was 25% for the 16 dogs in the study (three complete responses and 1 partial response). A diffuse lymphoplasmacytic infiltrate was present within tumors from the responding dogs, while this change was not observed in tumors from nonresponders.

Liposome-encapsulated muramyl tripeptide

Liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) is derived from a short structural unit of the *Mycobacterium* cell wall. Enclosing the bacterial wall component within a lipophilic liposome permits efficient *in vivo* targeting of monocytes and macrophages. When L-MTP-E is taken up via phagocytosis, inflammatory cytokines such as TNF-α and IL-6 are produced, leading to monocytes/macrophage activation with enhanced tumoricidal ability. The antitumor efficacy of this approach, especially against metastatic disease, has been well documented in phase II and III clinical trials in people with metastatic melanoma, osteosarcoma, and renal cell carcinoma.[84–87]

In veterinary medicine L-MTP-PE has been evaluated in several clinical trials in dogs and cats with various malignancies.[88–93] The survival benefit of L-MTP-PE therapy has been most clearly demonstrated in dogs with appendicular OSA. In one of the earlier clinical trials, dogs were randomized to receive either L-MTP-PE (i.v.) or a placebo control following limb amputation.[94] Dogs receiving L-MTP-PE had a median survival time of 7.4 months compared to 3 months for placebo treated dogs. But because 70% of dogs in both arms of the study succumbed to metastatic disease within a year, subsequent studies have evaluated L-MTP-PE therapy in conjunction with chemotherapy in the postoperative setting. Dogs randomized to L-MTP-PE following four doses of cisplatin (70 mg/m² q 4 weeks) had a median survival time of 14.6 months (p<.05) versus 10 months in dogs receiving cisplatin alone.[90]

The efficacy of L-MTP-PE has been assessed in canine hemangiosarcoma.[89] Dogs with splenic HSA, without evidence of metastasis postsplenectomy, received either doxorubicin/cyclophosphamide + L-MTP-PE or doxorubicin/cyclophosphamide therapy and empty placebo liposomes (L-PE). Dogs receiving L-MTP-PE had a significantly increased survival time of 9 months compared to 5.7 months for dogs treated with chemotherapy and placebo liposomes. In a study of dogs and cats with mammary adenocarcinoma, however, no significant increase in the disease free interval or survival time was found.[88,93]

Clinical benefit of combination therapy with L-MTP-PE and recombinant canine granulocyte macrophage colony-stimulating factor (rcGM-CSF) has also been assessed.[92] GM-CSF is a hematopoietic growth factor that enhances production of granulocytes, macrophages and eosinophils. Dogs with oral melanoma were randomized to receive either the combination of L-MTP-PE + rcGM-CSF or L-MTP-PE alone. When compared with historical controls (dogs treated with surgical resection alone), no benefit for dogs with stage II or III disease was seen in either arm of the study. For dogs with stage I disease, however, survival times were significantly increased in the L-MTP-PE treatment arm. This study did not demonstrate any therapeutic advantage of rcGM-CSF over L-MTP-PE alone.

Liposome-DNA complexes

The ability of bacterial DNA (e.g., plasmid DNA) and CpG oligonucleotides to elicit activation of innate immune responses can be markedly enhanced when the DNA is complexed to cationic liposomes.[95–98] This potentiation of immune activation is thought to occur in part due to protection of the DNA from degradation. However, the major role of cationic liposomes is likely to be the targeting of DNA into the endosomal compartment, where the receptor for bacterial DNA and CpG oligonucleotides (Toll-like receptor 9) is primarily expressed.[99,100] Studies of liposome-DNA-complexes (LDC) immunotherapy in mouse tumor models have demonstrated induction of strong antitumor activity, which appears to be mediated largely through induction of NK cell activity and release of IFN-γ.[96] In addition, we have also shown recently that LDC are also potent stimulators of type I interferon release in mice.[101]

The therapeutic potential for use of LDC in tumor immunotherapy in dogs has also been demonstrated. In one study, it was shown that intravenous delivery of LDC encoding the IL-2 gene in dogs with stage IV osteosarcoma elicited potent immune activation and NK cell activity.[102] In addition, treatment was associated with a significant increase in survival times compared to historical controls. A second study in dogs with soft tissue sarcomas demonstrated that intravenous infusion of LDC was capable of eliciting antitumor activity, mediated in part by angiogenesis inhibition and by direct antitumor effects.[103] Thus, LDC have potential as a new approach to tumor immunotherapy in dogs through induction of innate immunity.

Recombinant Cytokine Therapy

Interleukin 2 (IL-2)

IL-2 was discovered in the late 1970s as a growth factor for T cells.[104] IL-2 is produced by activated T cells following recognition of antigen-MHC and costimulatory molecules on the APC cell surface. IL-2 induces clonal expansion of antigen specific T cells and activates APCs such as DCs, macrophages, and B cells. This leads to systemic production of numerous inflammatory cytokines including IFN-γ, TNF-α, and IL-6, and strong induction of both innate and adaptive immunity. IL-2 also has pronounced stimulatory effects on natural killer and lymphokine-activated killer (LAK) cells (discussed further in Section IV). These multiple immunostimulatory effects make IL-2 an attractive candidate for immunotherapy.

Early experiments in tumor-bearing mice demonstrated the dose-dependent effect of intraperitoneal IL-2 administration in slowing the growth of pulmonary metastases secondary to several types of cancer.[105,106] High-dose therapy (>100,000 units of rIL-2) was found to decrease the number of pulmonary metastases by more than 80% in mice with colon adenocarcinoma and melanoma. In a model of disseminated leukemia, twice-daily IL-2 therapy cured more than 50% of the mice when administered for 5 days beginning a week after injection of neoplastic cells.[107]

Unfortunately the dramatic antitumor properties of IL-2 therapy were also associated with significant toxicity in mice and humans. IL-2 toxicity correlates directly with dose and duration of administration and can impact every organ in the body. IL-2 causes pronounced capillary leakage (manifested as pulmonary edema, pleural effusion, and ascites), as well as hepatocellular necrosis, renal dysfunction, dermatitis, anemia, and thrombocytopenia.[108–110] In people, high-dose intravenous IL-2 is associated with the most frequent and severe side effects, but less toxic regimens using lower doses and other routes of administration also result in significant toxicity.

To avoid the toxicity of systemic therapy, many investigators have evaluated alternative methods of IL-2 delivery. Direct intratumor injection slows the growth of a number of tumor types.[111–113] Other approaches include transfection of IL-2 into tumor cells or the use of antibody-IL-2 fusion proteins that specifically target neoplastic cells through known tumor antigens.

Systemic IL-2 therapy is frequently combined with other treatment modalities such as chemotherapy, radiation therapy, adoptive immunotherapy, and tumor vaccines. The goal of combination therapy is to increase the antitumor effectiveness of the primary treatment and permit lower doses of adjunctive IL-2 therapy so that toxicity can be reduced or avoided.

There are a large number of human clinical trials for IL-2, mostly for the treatment of metastatic melanoma, advanced renal cell carcinoma, and hematologic malignancies such as lymphoma and leukemia. The overall response (complete and partial remissions) rate for these trials hovers around 20%, but people that do respond have a high frequency of long-term, durable remissions.[114] In a clinical trial for melanoma, cisplatin-based chemotherapy combined with low-dose IL-2 yielded an overall response rate of 18%; however, three of the six responders had remission times greater than 3 years. When IL-2 is combined with other cytokines such as IFN-γ or with cytotoxic chemotherapy, overall responses are as high as 40% to 50%.[115,116] Given the advanced disease in most of these patients, this response rate is highly significant.

In veterinary medicine, treatment of cancer with human recombinant IL-2 (hrIL-2) holds promise in several species. Helfand et al. demonstrated that canine lymphocytes proliferate in response to hrIL-2, indicating that the canine IL-2 receptor can respond to the human cytokine.[117] The dogs in this study received high dose, i.v. IL-2 therapy for 4 consecutive days; mild gastrointestinal toxicity was noted in all dogs, but fever and more serious problems did not occur. The effects of longer term systemic therapy were not evaluated. In a separate study, canine lymphokine activated killer cells were induced *in vitro* in response to IL-2; interestingly the investigators noted that LAK cells obtained from tumor-bearing dogs demonstrated decreased cytotoxicity against a canine thyroid tumor cell line compared to LAKs isolated from healthy dogs.[118]

Toxicity and efficacy of liposome-IL-2 targeted to the lungs by inhalational delivery was evaluated in dogs with pulmonary metastases and primary lung carcinoma.[119,120] Two of four dogs with metastatic pulmonary osteosarcoma had complete regression of metastases, while the other two dogs had lesions that remained stable for more than 12 months. Lytic activity of lymphocytes obtained from bronchoalveolar lavage was significantly increased after 15 days of therapy. Toxicity was minimal for all nine dogs in the study. The canine trial led to a similar phase I study for people with pulmonary metastases from a variety of primary tumors.[121] Nine patients received nebulized liposome-IL-2 three times daily in an outpatient setting. No significant toxicity was observed; three patients achieved durable stable disease.

Interleukin 12 (IL-12)

IL-12 was initially identified in 1989 as a NK cell stimulatory factor and cytotoxic lymphocyte maturation factor.[122] Similar to IL-2, IL-12 has pronounced stimulatory effects on both the innate and adaptive immune system. IL-12 is produced primarily by DCs, macrophages, neutrophils, and B cells. IL-12 increases IFN-γ production from NK and T cells and enhances their lytic activity. IL-12 provides a strong stimulus to DCs, enhancing DC maturation and driving development of a Th1 cellular immune response. Additionally, IL-12 induces tumor cell production of IP-10 and Mig, both of which are potent antiangiogenic chemokines.

Phase I clinical trials in humans with systemic IL-12 revealed severe side effects that were not predicted from mouse models. The median tolerated dose of systemic administration of IL-12 was found to be 2 to 3 orders of magnitude lower than that in the mouse.[123] Not surprisingly, this finding dampened initial enthusiasm for IL-12 therapy. Targeted gene delivery of IL-12 appears more promising than systemic administration; this approach significantly extended survival in people with breast cancer, head and neck cancer, and melanoma.[123] Interestingly, antitumor effects were not limited to the injected tumor in some responding patients, suggesting that local therapy can induce a systemic antitumor immune response without eliciting toxicity.

Clinical efficacy of IL-12 has not been assessed in veterinary oncology. Preclinical work by Akhtar et al. utilized a canine hemangiosarcoma cell line engrafted onto immunocompromised mice to establish an *in vivo* model of angiogenesis.[124] In this model, IL-12 demonstrated strong antiangiogenic and antitumor effects. The investigators also evaluated the efficacy of an IL-12 fusion protein created by coupling recombinant IL-12 to αvβ3 integrin, which is expressed on rapidly dividing endothelial cells.[125] Targeted delivery of IL-12 to the tumor microenvironment prevented tumor angiogenesis at concentrations well below those needed to demonstrate the antiangiogenic activity of recombinant IL-12 alone. Although phase I studies need to be performed, IL-12 may prove to be a valuable anticancer therapeutic in animals, especially when used to treat locally confined disease.

Interleukin 15 (IL-15)

IL-15 was identified in 1994 based on its ability to mimic IL-2 induced T cell proliferation.[126] Subsequent work revealed many additional similarities between the two cytokines including the use of common signaling components and stimulation of NK and LAK cells. It is the difference between the two cytokines, however, that holds the most promise in the application of IL-15 to the treatment of cancer.

Initially, both IL-2 and IL-15 promote proliferation of CD4+ and CD8+ T cells. Continued stimulation with IL-2, however, leads to activation-induced cell death of CD4+ T helper cells, while repeated IL-15 administration blocks this effect and sustains CD4+ T cell proliferation.[127] IL-15 also selectively stimulates memory CD8+ T cells and is critical for their long-term maintenance.[128] Perhaps most significant is the finding that IL-2 supports the growth of regulatory T cells and may in fact be a better growth factor for this suppressor T cell phenotype than for other types of T cells[4]. So far, IL-15 has not been implicated in regulatory T cell function or survival and may therefore permit CD4+ and CD8+ T cell expansion without simultaneous stimulation of Tregs.

Efforts are under way to begin clinical evaluation of IL-15 in people with renal cell cancer and metastatic melanoma. IL-15 is also being assessed in preclinical trials for use as a vaccine adjuvant.[129] Toxicity studies are encouraging; in mice low doses of IL-15, unlike IL-2, triggers efficient NK cell activation and T cell proliferation without induction of toxicity. The application of IL-15 to veterinary species is also just beginning: feline IL-15 had been cloned from the lymph node of a cat infected with feline infectious peritonitis virus and appears to support proliferation of feline lymphocytes *in vitro*.[130]

Interferons

The interferons (IFNs) were initially characterized based on their secretion following viral infection.[131] Besides possessing potent antiviral activity, the interferons play a central role in the immune response to numerous other pathogens and to tumors. Their antitumor activity stems from both direct and indirect inhibitory effects on cell proliferation, induction of apoptosis, enhancement of CTL responses, and pronounced antiangiogenic effects.[132] The interferons are divided into two distinct classes: the type I IFN family, consisting of IFN-α and IFN-β, and type II, limited to IFN-γ.

Type I IFNs. The type I IFNs exert strong antiproliferative effects through multiple cell signaling pathways. For example, IFN-α and IFN-β inhibit c-myc expression and phosphorylation of retinoblastoma protein, both of which are important proteins in cell cycle control. IFN-α induces apoptosis by interacting with the Bcl-2/Bax and the TNF/Fas receptor families of proteins that regulate programmed cell death. The Type I IFNs are clinically effective angiogenic inhibitors; this has been most clearly demonstrated in the successful treatment of children with hemangioma.[133] Antiangiogenesis is largely mediated through downregulation of tumor-induced vascular endothelial growth factor and basic fibroblast growth factor production.[132,134]

The Type I IFNs have been evaluated in phase II and III clinical trials for the treatment of a variety of human malignancies including melanoma, multiple myeloma, renal cell carcinoma, leukemia, and a wide range of epithelial malignancies.[131] The best response rates are seen in treatment of renal cell carcinoma and in melanoma, where single agent IFN-α or IFN-α-2b therapy improves disease-free survival in patients with metastatic disease.[135,136] Clinical trials evaluating combination of IFN-α or IFN-β with various chemotherapy protocols have not demonstrated a benefit over chemotherapy alone; this finding was surprising given clear *in vitro* synergy between type I IFNs and chemotherapy drugs such as vinblastine and dacarbazine during preclinical testing.[131] Increased frequency and severity of toxicity occurred in many of these trials, making justification of type I IFN therapy difficult in light of low overall response rates.

Application of type I IFNs to veterinary medicine has been best studied in feline infectious diseases such as FIV, FIP, and FeLV. In one study, adoptive transfer of IL-2 activated T cells into FeLV-infected cats was combined with rhu-IFN-α-2b and zidovudine (AZT) therapy.[137] This protocol resulted in durable resolution of viremia in the majority of treated cats.

Type II IFN (IFN-γ). The antitumor properties of IFN-γ involve many of the same effects on cell cycle proteins as the type I IFNs as well as important stimulatory effects on immune effector cells. IFN-γ is the major physiologic activator of macrophages and therefore plays a critical role in induction of the innate immune response. Activated macrophages demonstrate efficient tumor cell killing; this is mediated largely through the cytotoxic effects of nitric oxide (NO). IFN-γ enhances expression of MHC class I and II molecules on a wide variety of cells, including tumors, increasing the efficiency of antigen recognition and processing by effector T cells.

Preclinical work in veterinary medicine by Kurzman et al. demonstrated increased *in vitro* tumoricidal activity of pulmonary alveolar macrophages against a canine osteosarcoma cell line following exposure to recombinant canine IFN-γ.[138] A separate study showed increased antigenicity of canine mammary and melanoma tumor cell lines following treatment with canine IFN-γ.[139] MHC class I and II expression on the tumor cell surface was increased by 20- to more than 100-fold depending on the cell line evaluated, suggesting that IFN-γ treatment could increase recognition of tumor cells by DCs and effector T cells.

Despite the *in vitro* evidence for the immunostimulatory potential of IFN-γ, human clinical trials for treatment of many different solid and hematologic tumors have been disappointing. One of the more successful trials was treatment of chronic myelogenous leukemia where a low rate of complete tumor regression was noted in patients with advanced disease.[140]

CANCER VACCINES

The goal of cancer vaccine therapy is to elicit an antitumor immune response that results in clinical regression of a tumor or its metastases. Unlike traditional cytotoxic chemotherapy, which results in rapid tumor cell death, clinical response to vaccine therapy depends on the development of an adaptive immune response that may take several months or more to appear. Furthermore, it is becoming increasingly evident that a demonstrable cell-mediated or humoral antitumor immune response, even a strong one, does not necessarily correlate with clinical tumor regression. The observation of "mixed responses" is another frequent problem in assessing treatment outcome; this phenomenon is characterized by the differential response of metastases within different tissues of the same patient. Factors such as these pose significant challenges to the design of clinical trials, especially in terms of monitoring efficacy and making comparisons between trials. To overcome these obstacles, a new set of criteria was recently proposed by the National Cancer Institute. Termed RECIST (response evaluation criteria in solid tumors), an objective clinical response is now defined as a 30% reduction in the sum of the maximum diameters of lesions as well as the appearance of no new or progressive lesions.[20]

There are numerous different types of tumor vaccines in phase I through III trials, and many have been evaluated for efficacy across a wide range of tumor types. It is beyond the scope of this chapter to cover all of these approaches and their protocols in detail; we will focus instead on those that have been most successful in human oncology and are most readily applicable to veterinary cancer patients.

Tumor-Associated Antigens

Tumors are detected by the immune system through specific tumor-associated antigens (TAAs) that are recognized by both CTLs and antibodies. TAAs can be unique to an individual tumor or common to a particular tumor type. Unique TAAs arise from mutated cellular gene products such as p53, ras, and p21 and are often secondary to chemical or radiation-induced damage. These antigens elicit a stronger immune response (are more immunogenic) than shared tumor antigens but do not make practical therapeutic targets for cancer immunotherapy because of their narrow specificity.

Many of the shared tumor antigens are normal cellular antigens (arising from self tissues) that are overexpressed in tumor tissues. The first group to be identified is termed cancer testes antigens, and they are expressed in normal testes, melanoma, and various forms of carcinoma. Important antigens in this category include the MAGE gene family and NY-ESO-1, and they generally induce both antibody and tumor-specific CTL

responses. Another large group is the melanocyte lineage proteins that are expressed on normal melanocytes as well as tumor cells. These antigens, including gp100, MART-1/Melan-A, and tyrosinase related protein-1 (TRP-1), generate strong tumor-specific CTL responses.

Approaches to Tumor Vaccines

Whole-cell and tumor-cell lysate vaccines

The preparation of whole-cell or tumor-cell lysate vaccines is straightforward and represents one of the simplest approaches to cancer vaccine therapy. These vaccines can be made from a patient's own tumor tissue (an autologous vaccine) but are more commonly prepared from individuals within the same species bearing the same type of cancer (an allogeneic vaccine). Whole-cell vaccines generally contain multiple tumor cell lines that have been γ-irradiated so that they are no longer functionally active. Adjuvants are commonly added either before or after irradiation. Tumor-cell lysate vaccines consist of mechanically lysed tumor cells or tumor tissue and are usually combined with an adjuvant. Unlike vaccines derived from specific peptides or purified proteins, the polyvalent nature of the whole-cell and lysate vaccines covers a range of potential tumor-associated antigens without requiring their individual identification. These vaccines also protect against heterogeneity in tumor antigen expression, a documented form of tumor evasion in several types of cancer.

The authors have investigated the potential therapeutic role of an allogeneic tumor-cell lysate vaccine in a nonrandomized phase I/II trials in dogs with either melanoma or hemangiosarcoma.[141,142] The melanoma or hemangiosarcoma lysates were adjuvanted with a lipid-DNA complex, an adjuvant that elicits a strong cell-mediated immune response to delivered antigens.[143] Client-owned dogs received the tumor vaccine once every 2 weeks for 5 treatments then once monthly for at least 3 months. The dogs were allowed to continue with standard of care therapy for their disease. Toxicity was minimal in both studies with only 6 of 75 dogs experiencing nausea, transient fever, or mild to moderate abdominal pain. Determination of overall efficacy for the trials was complicated by the use of multiple treatment protocols in addition to the tumor vaccine, but median survival of vaccinated dogs with hemangiosarcoma was significantly longer than a control population.

Besides the use of different adjuvants, whole-cell, or lysate vaccines may be modified in a number of ways to enhance antitumor immunity. Immunostimulatory cytokines such as IL-2, IL-12, GM-CSF, and IL-15 are most frequently used. In a clinical trial for dogs with advanced-stage soft tissue sarcoma or melanoma, an autologous whole-cell vaccine, transfected with human GM-CSF, was prepared for each of 16 dogs.[144a]

Objective tumor responses were seen in 3 of these dogs, including regression of primary tumors as well as metastatic lesions. Histopathologic examination of vaccine sites in the dogs receiving the vaccine demonstrated an intense inflammatory cellular infiltrate not seen at control (placebo) vaccine sites.

In another trial in dogs with malignant melanoma, an allogeneic whole-cell vaccine expressing xenogeneic (human) gp100 (a commonly used melanoma differentiation antigen) was evaluated in the context of a phase II clinical trial.[144b] Of 34 dogs with measurable malignant melanoma in trial, an overall response rate of 17% (1 complete response and 5 partial responders) was observed, and a tumor control rate (complete and partial response and stable disease of >6 weeks duration) of 35% was observed. While overall delayed-type hypersensitivity and *in vitro* T cell cytotoxicity to whole tumor cells was enhanced following vaccination, only the former correlated with clinical outcome.[144b]

Another whole-cell vaccine study investigated treatment of dogs with B cell lymphoma.[145] After induction of remission with a standard 19-week CHOP-based chemotherapy protocol, dogs were randomized to receive a human GM-CSF-autologous whole-cell lymphoma vaccine or placebo injections. Although no significant difference in the median length of the first remission was observed, dogs receiving the tumor vaccine experienced significantly increased survival compared to control dogs.

Genetic vaccines for cancer

Vaccines based on immunization with plasmid DNA are designed to elicit antigen-specific humoral and cellular immunity against a known tumor target. Although identification of tumor-associated antigens is more laborious than using whole-cell or lysate vaccines, plasmid DNA is relatively simple and inexpensive to purify, and the immunogen of interest can be readily cloned into a bacterial expression plasmid with a constitutively active promoter. The vaccine is generally given intradermally, or into the muscle, where the plasmid vector is expressed, leading to antigen uptake by local DCs. The DCs then present the antigens in both the MHC class I and II processing pathways, along with the proper costimulatory molecules. This "natural" approach greatly facilitates the induction of tumor-specific CTL responses.

Another advantage of using plasmid DNA vaccines is the inherent immunogenicity of the bacterial plasmid vector. This is due to the presence of CpG motifs, unmethylated dinucleotide-CpG residues, which occur much more frequently in bacterial DNA than mammalian DNA. The repeated CpG dinucleotides are recognized as a danger signal by DCs with subsequent triggering of a predominately Th1-type immune response.[146]

Plasmid DNA vaccines are currently being evaluated in human patients with melanoma, lymphoma, prostate cancer, and renal cell carcinoma. One of the earliest clinical trials using plasmid DNA was conducted in the early 1990s in patients with metastatic melanoma to skin and other sites.[147] In this trial, patients received intralesional injections of plasmid DNA vaccine encoding a foreign human MHC class I gene, HLA-B7, complexed with liposomes. This strategy was designed to enhance the immunogencitiy of tumor cells through immune activation by the foreign MHC antigen. Although 100% of the patients demonstrated tumor-specific CTLs in peripheral circulation following vaccination, only 20% experienced significant tumor regression.

A more promising clinical trial was recently completed in dogs with malignant melanoma.[148] The investigators immunized dogs with human DNA encoding tyrosinase, a melanoma differentiation antigen essential in melanin synthesis. The use of a xenogeneic vaccine takes advantage of the immunogenicity of foreign DNA and is an effective approach to the induction of antibodies and cytotoxic T cells specific for the tumor-associated antigen.[149] The nine dogs of this study received the xenogeneic DNA vaccine biweekly for 2 weeks and did not receive any other treatments during the course of vaccination. All of the dogs had advanced disease (World Health Organization, stage II, III, or IV) at the start of the study; one dog with multiple pulmonary metastases experienced complete remission lasting 329 days, and two other dogs with stage IV disease had long-term survivals of greater than 420 days. The overall response rate was 44% with a 389-day median survival, and toxicity was limited to mild pain at the vaccine injection site.

A method combining both a genetic and a whole-cell vaccine approach was recently applied in dogs with malignant melanoma.[144b]

Viral-vectored tumor vaccines

Viral vectors are useful tools for the expression of genes within various host cells, particularly professional antigen presenting cells. For cancer patients, viruses are often designed to deliver genes encoding tumor-associated antigens or immunostimulatory cytokines and molecules. Attenuated or replication-defective viruses are especially attractive vectors since they can be designed to efficiently infect host cells and elicit innate and adaptive immune responses against the virus, but are themselves unable to divide and spread within the host.

The Poxvirida family contains several viruses that are being used to treat humans and animals with cancer. This family of DNA viruses is especially amenable to vaccine therapy because large amounts of foreign DNA and multiple genes can be stably inserted within viruses such as vaccinia or the avipoxviruses and then reliably

expressed within infected host cells. Another advantage is the inflammatory nature of some of the poxvirus viral proteins; genes that encode for weakly immunogenic proteins, such as carcinoembryonic antigen (CEA), for example, become much more immunogenic when delivered in a recombinant vaccinia virus vaccine.[150,151]

Several phase I clinical trials have been completed in human cancer patients with either recombinant vaccinia virus vaccines or with replication-defective avipoxvirus vaccines such as the canarypox virus ALVAC. In a number of trials, the viral vector is designed to deliver both a tumor-associated antigen and a costimulatory molecule or cytokine. ALVAC with CEA and CD80 (a key costimulatory molecule expressed on DCs) was tested in patients with CEA-expressing adenocarcinoma.[152,153] In these studies, increased tumor-specific CTLs were generated as well as significant leukocyte infiltration and CEA antigen expression at vaccine sites. No toxicity was associated with vaccination; protocols generally consisted of four to eight weekly or biweekly vaccines followed by monthly boosters. Phase 2 trials are presently under way for prostate cancer and melanoma as well as adenocarcinoma.[154]

The canarypox virus vector ALVAC and an attenuated vaccinia virus vector NYVAC, have been evaluated in veterinary patients. In a preliminary study, either ALVAC or NYVAC was administered intralesionally to dogs with melanoma.[155] Because the vaccines were engineered to express the luciferase gene, the biodistribution of the vectors could be mapped within the tumor. Expression of viral particles was confined to the tumor itself and did not extend to adjacent normal tissues or draining lymph nodes. Based on this preliminary study, a clinical trial in cats with fibrosarcoma was conducted.[155] An ALVAC recombinant virus expressing the feline IL-2 gene was constructed as well as a NYVAC virus expressing human recombinant IL-2. All cats in the study were treated initially with surgical tumor resection (debulking) followed by iridium-based radiotherapy. A total of 36 cats were treated intralesionally with one or the other of the virus vector vaccines, and an additional 18 cats in the control group did not receive either vaccine. In the absence of immunotherapy (treatment with surgery and iridium alone), tumor recurrence was 61% over a 1-year follow-up period. In contrast, only 28% of cats receiving either vaccine demonstrated tumor recurrence during the same period.

Dendritic cell vaccines

The potent antigen-presenting capabilities of the mature DC and its role in initiation of an adaptive immune response make manipulation of this cell a logical choice for cancer immunotherapy. DCs can be "pulsed" or loaded with a variety of substances including tumor antigen peptides, RNA or DNA of known tumor antigens, tumor lysates, and parts of apoptotic tumors. DCs can be transfected directly or modified through viral and nonviral vectors (such as liposomes). Alternatively, DCs can be injected directly into tumors or adoptively transferred to cancer patients.

The preparation of DC vaccines is a labor-intensive process.[156,157] DCs are typically isolated from the peripheral circulation of human patients, exposed to the antigen or gene of interest, matured in tissue culture using a combination of recombinant cytokines, and then reintroduced into the patient. This *ex vivo* process takes about 7 to 10 days and must be done in a sterile laboratory environment. Strategies to deliver antigens directly to DCs *in vivo* are under investigation; an example of one approach is the targeting of DCs with single chain antibody fragments (similar to Fab fragments) linked to antigen bearing liposomes.[158] It is also possible to identify subsets of DCs based on their differential expression of cell surface molecules, including lectins, integrins, and costimulatory molecules.[159] These markers may permit specific *in vivo* targeting of subsets of DCs and allow improved control of the antitumor immune response.

Clinical trials with DC vaccines generally have higher response rates than trials with other cancer vaccine types including peptide, viral vectors, and tumor-cell/lysate vaccines.[160] Because of the time and cost associated with DC vaccine preparation, however, patient numbers in most of these trials are small (usually less than 20). In a clinical trial in humans with B cell lymphoma, DCs pulsed with idiotype immunoglobulin from autologous lymphoma were adoptively transferred to patients. All four people in this study developed significant antitumor CTL responses; two patients demonstrated complete tumor regression, while one had a partial remission, and the other experienced progressive disease.[161] In a separate trial in people with advanced melanoma, DCs pulsed with tumor lysate or a cocktail of melanoma peptide antigens elicited an objective overall response rate of 30%.[162] Many clinical trials are still in progress, most with the goal of optimizing route of vaccine administration, methods of preparing DCs, and the role of concurrent cytokine therapy or chemotherapy in enhancing vaccine efficacy.

Gyorffy et al.[163] assessed the efficacy of DC vaccination in dogs with oral melanoma. Bone-marrow derived DCs from three tumor-bearing dogs were expanded *ex vivo*, then transduced with human gp100 using an adenovirus vector. Three vaccines were administered subcutaneously once monthly; dogs were treated prior to vaccination with conservative tumor resection and coarse-fraction radiation. One dog had a measurable tumor-specific CTL response (to human gp100) with no evidence of tumor at 48 months after treatment. Another dog did not demonstrate tumor-specific

cytotoxic T cells but remained disease free for 22 months. However, both of these long-term survivors had stage I tumors.

TUMOR IMMUNOTHERAPY WITH MONOCLONAL ANTIBODIES

The concept of using monoclonal antibodies (MAbs) to specifically target and treat cancer has been studied for more than 30 years. The technology that paved the way for this approach was originally developed by Kohler and Milstein in 1975.[164] They developed the "hybridoma": cell lines capable of producing monoclonal antibodies derived from antibody-producing B cells hybridized with a murine myeloma cell line. From this foundation, monoclonal antibody therapy has grown to encompass the treatment of a wide range of malignancies with targeted delivery of toxins, radionuclide drugs, and cytokines. Since the mid-1990s, therapeutic monoclonal antibodies have emerged as one of the most rapidly growing classes of drugs, with 17 antibodies in use today approved by the Food and Drug Administration, eight of them for malignant diseases.[165] Table 13-1 lists these MAbs in order of FDA approval.

Initially, human clinical trials used mouse MAbs, but success was limited by several factors. One key problem was the induction of human antimouse antibodies. These antibodies inactivate the mouse MAbs and can potentially trigger allergic or anaphylactic reactions that limit repeated MAb administration. Additionally, ADCC is impaired by structural differences between the Fc portion of the mouse immunoglobulin and the Fc receptors on human immune effector cells. Another obstacle is antibody production itself; developing antibodies specific for each individual patient is labor intensive, costly and largely impractical from a pharmaceutical standpoint.

To circumvent these problems, modern MAb therapy relies on genetically engineered antibodies. Chimeric MAbs combine the variable region of the mouse antibody with the constant domains of the human immunoglobulin molecule. Chimeric MAbs are approximately two-thirds human and are consequently much less immunogenic, prolonging serum half-life and allowing repeated administration.[165]

A newer refinement of this approach is the development of humanized antibodies, which are 95% human. They are constructed by grafting the murine hypervariable regions of the immunoglobulin structure onto the human antibody molecule. Fully humanized antibodies are also possible; they are readily generated in mice that have been transfected with human immunoglobulin genes and then vaccinated with desired antigens.[166] Humanized antibodies demonstrate markedly improved ADCC against human mononuclear cells as well as more stable pharmokinetics; these factors dramatically improve efficacy and decrease toxicity.[167]

Unconjugated Monoclonal Antibodies

Anti-CD20 (Rituximab)

One of the first MABs approved for people with cancer is Rituximab, commonly used to treat non-Hodgkin's lymphoma. Rituximab is a chimeric IgG1 monoclonal antibody directed against CD20, a transmembrane protein highly expressed on the majority of B cell lymphomas. CD20 is an example of a nearly ideal antigen for targeted therapy because expression is limited to mature B cells and is not found on immature progenitor cells or plasma cells. Additionally, CD20 is stably expressed on the B cell surface and does not undergo internalization or shedding with antibody binding occurs.[165]

Rituximab binding causes destruction of the CD20-expressing cell through several mechanisms including antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity and direct induction of apoptosis.[168] Clinically, administration of rituximab causes rapid systemic depletion of normal and malignant CD20 expressing B cells that persists for 3 to 6 months. Initial infusion is commonly associated with transient flulike symptoms (fever, nausea, chills), which can be alleviated by pretreatment with antihistamines and antiinflammatory drugs. These symptoms are infusion related and not specific to rituximab itself; some of these side effects may also be associated with rapid tumor-cell lysis.[169]

Rituximab therapy is FDA-approved for the treatment of relapsed or refractory low-grade NHL. This is based on several large clinical trials that demonstrated an overall response rate of 50% (6% complete remission [CR] and 44% partial remission [PR]) with stable disease duration of approximately 12 months.[165,170,171] The response to a second course of rituximab therapy when successfully treated patients relapse is similar, with an overall response of 40% and an often longer time to progression (17 months) than after the first course of treatment.[172] Combining rituximab with chemotherapy yields synergistic results: one trial demonstrated a 95% response rate (55% CR, 40% PR) in previously untreated lymphoma when rituximab was given concurrently with CHOP-based chemotherapy. Median time to progression and duration of remission were significantly prolonged than in patients treated with CHOP chemotherapy alone.[173] Canine lymphoma cells do not express a cross-reacting CD20; this and the problem of dogs mounting

TABLE 13-1   Monoclonal Antibodies in the Order They Were Approved by the FDA

FDA Approved	Product	Target	Type	Indication
1986	Muromonab (Orthoclone OKT®)	CD3	Murine IgG2a	Transplant rejection
1994	Abciximab (Reopro®)	GPIIb/IIIa	Chimeric Pab	Prevention of re-stenosis after PTCA
1997	Daclizumab (Zenapax®)	CD25	Humanized IgG1	Transplant rejection
1997	Rituximab (Rituxan®)	CD20	Chimeric IgG1	B-cell lymphoma
1998	Basiliximab (Simulext®)	CD25	Chimeric IgG1	Transplant rejection
1998	Palivizumab	RSV	Humanized IgG1	RSV bronchiolitis
1998	Infliximab (Remicade®)	TNF	Chimeric IgG1	Crohn's disease, rheumatoid arthritis
1998	Trastuzumab (Herceptin®)	HER2	Humanized IgG1	Breast cancer
2000	Gemtuzumab (Mylotarg®)	CD33	Humanized IgG4-toxin-conjugate	Acute myeloid leukemia
2001	Alemtuzumab (MabCampath®)	CD52	Humanized IgG1	Chronic lymphatic leukemia
2002	64Y-ibritumomab (Zevalin®)	CD20	Murine IgGI-radionuclide-conjugate	B-cell lymphoma
2002	Adalimumab (Humira®)	TNF	Human IgG1	Rheumatoid arthritis
2003	Omalizumab (Xolair®)	IgE	Humanized IgG1	Asthma
2003	131 I-tositumomab (Bexxar®)	CD20	Murine IgG-radionuclide conjugate	B-cell lymphoma
2003	Efalizumab (Raptiva®)	CDIIa	Humanized IgG1	Psoriasis
2004	Bevacizumab (Avastin®)	VEGFR	Humanized IgG1	Colorectal cancer
2004	Cetuximab (Erbitux®)	EGFR	Chimeric IgG1	Colorectal cancer

antihuman antibodies would make rituximab ineffective in the species.

Antiepidermal growth factor receptor antibody (Trastuzumab)

The human epidermal growth factor receptor 2 (HER-2, also called HER-2/neu) is a proto-oncogene that encodes the HER-2 receptor, a tyrosine kinase protein widely expressed on epithelial tissues. HER-2 is overexpressed in approximately 30% of adenocarcinomas of the breast, lung, gastrointestinal tract, and ovary.[174] HER-2 overexpression is typically 100-fold higher in malignant epithelium than in normal tissues. In women with breast cancer, overexpression correlates with decreased estrogen receptor levels and decreased survival.[165]

Trastuzumab (Herceptin) is a humanized antibody derived from a mouse MAb that recognizes an epitope on the extracellular domain of the HER-2 receptor. Although the mechanism of action of trastuzumab is not fully understood, binding of the antibody to its target results in downregulation of HER-2 expression. Cytotoxicity of tumor cells is partially mediated by ADCC and also results from the antiangiogenic activity of the MAb.[174]

Trastuzumab therapy appears most successful when combined with chemotherapy; this has been best studied in women with breast cancer. In a large phase III clinical trial involving 469 women with metastatic breast cancer, patients were treated with doxorubicin or epirubicin and cyclophosphamide alone or in combination with trastuzumab.[175] Women receiving the MAb demonstrated a higher response rate (50% versus 32%) than those treated with chemotherapy alone. In this trial, trastuzumab combined with chemotherapy prolonged median survival by nearly 5 months (from 20 to 25 months), even when given to women who initially failed chemotherapy and then crossed over to the MAb treatment arm of the protocol. *In vitro*, trastuzumab has a synergistic effect when combined with a variety of chemotherapy drugs including vinorelbine, cisplatin, and etoposide; several clinical

trials are in progress to evaluate various combination regimens.[176]

Antivascular endothelial growth factor antibody (Bevacizumab)

Targeting tumor vasculature and other components of the surrounding stroma is becoming an increasingly attractive approach to the immunotherapy of cancer. Reasons for this include applicability to various tumor types, increased stability of target antigen expression on nontransformed cells, and increased permeability of the stroma compared to solid tumor tissue. Bevacizumab is a humanized antibody against vascular endothelial growth factor (VEGF). Early in clinical evaluation, bevacizumab demonstrated synergistic effect when combined with certain chemotherapy drugs; this led to FDA approval of the MAb for first-line treatment of metastatic colorectal adenocarcinoma in combination with 5-fluorouracil.[177,178]

Bevacizumab was also evaluated in advanced stage renal carcinoma and breast cancer.[179,180] In these clinical trials, the antibody significantly increased response rates in patients that had previously failed multiple therapies but did not lead to increased overall survival. The addition of bevacizumab to standard chemotherapy protocols in the minimal disease setting may drastically improve the clinical outcome.

Monoclonal antibody 231

MAb 231 is a monoclonal antibody that recognizes canine lymphoma cells, although the precise target is not known. In preclinical work, Jeglum et al. demonstrated induction of ADCC against MAb 231-targeted canine lymphoma cells *in vitro*.[181] This was extended to a nonrandomized clinical trial in which dogs receiving MAb 231 in combination with chemotherapy were compared to dogs receiving chemotherapy alone (historical controls).[182] The median survival for dogs treated with the MAb was dramatically longer than dogs who received chemotherapy but not the MAb. Unfortunately, subsequent trials with the antibody were unable to confirm the results of the initial study, and the antibody is no longer available commercially.

Antiidiotype monoclonal antibodies

Enormous potential for diversity in antibody production arises during normal B cell development. Immunoglobulin variable-region gene segments frequently undergo recombination and somatic mutation, which results in an idiotype, an immunoglobulin sequence unique to each B cell. Antibodies directed against these idiotypes are referred to as antiidiotype. Monoclonal antiidiotype antibodies can be generated using hybridoma technology but must be customized for each individual patient. An advantage of this

approach is that antiidiotype MAbs are processed through both the class I and class II MHC pathways when taken up by APCs and elicit efficient activation of tumor-specific helper CD4 T cells and cytotoxic CD8 T cells.

Human clinical trials using antiidiotype MAbs include lymphoma, colorectal cancer, and melanoma. In a series of trials at Stanford University, 34 patients with relapsed or advanced stage lymphoma were treated with infusions of antiidiotype Mabs.[183–186] The overall response rate was 68% and included 6 CRs, most of which lasted more than 4 years. Patients who initially responded to antiidiotype therapy, but later relapsed, did so secondary to outgrowth of mutant idiotype-negative cells that were unable to bind the antiidiotype MAb. To circumvent this problem the investigators at Stanford generated antiidiotype antibodies coupled to keyhole limpet hemocyanin (KLH). This approach elicits a polyclonal immune response directed against multiple epitopes and thus limits the potential for mutant idiotype outgrowth of tumor cells. In a randomized clinical trial, significant increase in the disease-free interval occurred (8 years versus 1.3 years) when KLH-antiidiotype antibody combined with standard chemotherapy was compared to people treated with chemotherapy alone.[187]

Conjugated Monoclonal Antibodies

Immunotoxin Conjugated Antibodies

Immunotoxins are MAbs linked to bacterial, plant, or synthetic toxins. The idea of using tumor-specific antibodies to deliver toxins to tumors was first proposed in the early 1900s ago by Paul Ehrlich.[188] This "magic bullet" concept had to wait, however, for the hybridoma technology of Kohler and Milstein to put theory into practice. First-generation immunotoxins were made by chemically coupling toxins such as ricin (a plant toxin) or diphtheria toxin to MAbs. The early products were highly immunogenic and chemically unstable, sometimes falling apart before reaching the tumor target. These difficulties led to the development of recombinant immunotoxins that were more stably linked to the antibody until bound on the target cell surface.

Other important issues in immunotoxin design include selection of cell surface antigens that internalize on antigen binding. This allows release of the toxin within the cytosol of the target cell and prevents damage to untargeted tissues. Specificity for tumor cells versus normal cells is also critical; since toxins are designed to kill any cell that internalizes them, antigen expression on normal cells should be minimal.

Immunotoxins can be directed against both hematologic and solid tumors; however, treatment of

hematologic malignancies has generally been more successful. Difficulties in treating solid tumors include ineffective penetration of solid tumors with the large immunotoxin molecules and development of neurotoxicity due to cross-reactivity with cells of the central nervous system.[189]

One of the most successful immunotoxins is gemtuzumab-ozogamicin (GO), a humanized antibody against CD33 linked to calicheamicin, which is a potent cytotoxic antibiotic. CD33 is highly expressed on malignant blast cells in people with acute myeloid leukemia (AML) and myelodysplastic syndrome.[190] CD33 is also expressed in normal hematopoietic progenitor cells but is absent on pleuripotent stem cells and nonhematopoietic tissues. The clinical efficacy of GO was recently demonstrated in a large multicenter trial involving 142 people with relapsed AML.[191] The overall response rate was 30% with 16% CR and 14% PR. Mean remission duration was 7.2 months with patients achieving a CR and 4.4 months in those with a PR. The side effects of immunotoxin therapy are often more severe and more frequent than the mild flulike symptoms seen in standard MAb treatment. Side effects of GO therapy in the multicenter trial included moderate to severe thrombocytopenia, neutropenia, elevation of liver enzymes, and infusion-related reactions.

Other conjugated MAbs, especially those conjugated to ricin and *Pseudomonas* endotoxin, can directly damage normal endothelial cells. This is manifested as a vascular leak syndrome, characterized in humans by edema and weight gain. The narrow therapeutic window of the immunotoxins and the development of antitoxin antibodies limit the clinical benefit of this form of therapy. Work is in progress to decrease damage to normal tissues without loss of the potent antitumor cytoxicity delivered by immunotoxins.

Radionuclide conjugated antibodies

Radiolabeled MAbs are designed to deliver radioisotopes to tumor tissue while sparing unaffected organs and tissues. This approach improves on many of the weaknesses of the unconjugated MAbs. The energy released by radiolabeled MAbs penetrates more effectively into bulky solid tumors and is cytotoxic to tumor cells adjacent to the antibody-bound target cell (the "cross-fire" effect).

Therapeutic radionuclides can be broadly divided based on their emission characteristics into beta or alpha-particle emitters. Beta particle–emitting isotopes deposit low level radiation over relatively long distances (millimeter) and are typically used in patients with large tumor burdens and solid bulky tumors. Because of the long-range emissions of the beta particles, they generate considerable bystander cell killing; bone marrow toxicity is the primary side effect and is often

dose limiting. Alpha particle emitters, derived from the high-energy helium nuclei, are more potent than the lower energy beta particles and emit radiation over a much shorter path. Cytotoxicity induced by alpha particles is therefore more selective and damage to normal tissues, including bone marrow, is markedly less.[192]

As with immunotoxins, clinical experience with hematologic tumors such as lymphoma and leukemia is more extensive than for solid tumors. The radiation sensitivity of lymphoma and experience with anti-B cell antibodies led to the development of the first FDA-approved radiolabeled MAb, ^{90}Y-ibritumomab-tiuxetan (Zevalin) in 2002.[165] ^{90}Y-ibritumomab is a combination of the CD20 antibody ibritumomab (similar to rituximab) linked to tiuxetan, a chelating agent. It is a beta emitter with a narrow therapeutic window; myelosuppression typically appears 1 to 2 months after a single dose and persists for 2 to 4 weeks.

In a randomized phase 3 trial using ^{90}Y-ibritumomab in 143 patients with relapsed or refractory lymphoma, patients were treated with either a single dose of ^{90}Y-ibritumomab or with multiple doses of rituximab.[193] The overall response rate was 80% in the group receiving ^{90}Y-ibritumomab, compared to 56% in the rituximab arm. Interestingly, in a separate trial of 57 people refractory to rituximab therapy, 74% responded to ^{90}Y-ibritumomab.[194] This suggests that the mechanism of action of the radiolabeled antibody may be largely independent of its specificity for CD20.

Evaluation of radioimmunotherapeutics for other malignancies includes renal cancer, neuroblastoma, colonic adenocarcinoma, and breast cancer.[192] In many instances, the development of human antimouse antibodies occurs after administration of as few as two or three doses. Myelosuppression is usually the dose limiting toxicity, and damage to other organs is infrequent. Over the next several years, development of more specific therapeutics using different radionuclides, the use of radiation-dose modifying agents, and improved labeling techniques should lead to better agents for the radioimmunotherapy of cancer.

ADOPTIVE T CELL TRANSFER THERAPY

Simply put, the goal of adoptive cell transfer (ACT) therapy for cancer is to increase the antitumor properties of specific populations of effector cells in order to generate a more powerful and focused antitumor immune response. This is done by collecting peripheral immune effector cells from an individual patient, activating and expanding them in tissue culture *ex vivo*, then transferring the enhanced cell population back to the patient. Although the technological advances in

molecular immunology since the mid-1990s have made this approach feasible and relatively straightforward, the labor-intensive nature of ACT therapy limits its availability to large numbers of cancer patients.

Transfer of Lymphokine-Activated Killer Cells

In its earliest form, ACT therapy focused primarily on lymphokine-activated killer (LAK) cells, peripheral blood lymphocytes cultured in high concentrations of IL-2. LAK cells mediate potent tumor-cell lysis that is neither MHC restricted nor dependent on tumor type. Human LAK cells can easily be generated in tissue culture with recombinant IL-2 and expanded to high numbers without loss of activity. Their cytoxicity is not diminished in cancer patients compared to healthy people.[195,196]

Preclinical work in mice demonstrated the dramatic therapeutic effect of LAK-cell administration with concurrent IL-2 therapy using models of metastatic sarcoma, melanoma, and adenocarcinoma. Mice bearing pulmonary and hepatic metastases experienced significant tumor regression and increased survival.[197,198] In murine tumor models with liver metastases, IL-2 alone was moderately effective at high doses; when combined with LAK cells, however, and using a low dose of IL-2, mice demonstrated significant reduction in the number and size of liver nodules.[197]

Extension of mouse LAK/IL-2 studies to humans was disappointing. Despite some success in people with advanced melanoma, complete and durable responses to therapy were infrequent. The difficulties and expense associated with delivery of large numbers ($>10^{11}$) of LAK cells, in multiple cycles in addition to the significant toxicity of IL-2 administration, led to discontinuation of this form of therapy less than 10 years after the initial discovery of LAK cells.[199]

Application of adoptive transfer to veterinary species has been best studied in cats infected with FeLV or FIV. In cats with FeLV infection, lymphocytes were isolated from the popliteal lymph node of each animal and expanded in culture with a low dose of rh-IL-2 for 7 days.[200] LAKs were then reinfused into each cat as a single i.v. dose. Four of 16 cats completely cleared their viral infections based on detection of FeLV antigen, while 9 of the 16 cats experienced dramatic clinical improvement that persisted for more than 1 year.

Transfer of Tumor-Infiltrating Lymphocytes

The discovery in the 1980s of tumor-infiltrating lymphocytes (TILs) led to major improvements in ACT therapy.[201,202] The lymphocytes that traffic to and reside within tumors can be specifically isolated, activated, and expanded in culture with IL-2, similar to production of LAKs. Cultured TILs, however, exhibit 50- to 100-fold higher antitumor cytoxicity than LAK cells and can mediate both nonspecific LAK cell–like activity and specific autologous tumor recognition.[201] Infusion of TILs into mice with various types of metastatic cancer demonstrated therapeutic benefit; this effect could be further enhanced by combining TIL administration with systemic IL-2 and cyclophosphamide.

Subsequent experience with human TILs illustrates both their potential and limitations. Early clinical trials did not yield durable tumor regressions and revealed that not all TILs are created equally.[199] Characteristics such as the age of the TIL at the time of reinfusion, its doubling time, and the ability of the TIL to recognize and kill MHC-matched tumor cells were found to be important predictors of clinical outcome. TILs isolated from different types of cancer vary significantly in their ability to lyse tumor cells. The majority of TILs found within carcinomas of the breast, lung, and colon, for instance, are not tumor specific or have no functional activity. TILs derived from human melanomas, however, possess a high proportion of potent tumor-specific cytotoxic lymphocytes and are easier to derive in high numbers than TILs from other types of cancer.[203,204]

The superiority of TILs derived from melanoma has been clearly demonstrated. For example, Muul et al. reported that six of six TILs from melanoma patients effectively lysed autologous tumor cells but did not have activity against allogeneic tumors.[205] Subsequent studies demonstrated that melanoma TILs cultured in IL-2 could consistently produce CD8+ tumor-specific T cells that were readily expanded in culture and efficiently lysed tumor cells when given back to patients.

Besides the enhanced immunological function of melanoma-derived TILs, clinical response is markedly improved in the patients who receive them. The most promising clinical trials involve pretreatment of patients with nonmyeloablative chemotherapy using fludarabine and cyclophosphamide. These drugs deplete lymphocytes but preserve bone marrow stem cells. When transferred in this setting, the administration of melanoma-derived TILs along with systemic IL-2 therapy results in dramatic treatment responses. In one trial, 6 of 13 patients experienced significant tumor regression.[206] Additionally, 4 mixed-responses (i.e., regression of some lesions but growth of others) were seen. Persistence of the transferred cells was seen for 4 months after adoptive transfer. Similar results were also seen in a larger follow-up trial by these investigators. Tumor regression was observed in 18 (51%) of 34 patients treated with nonmyeloablative chemotherapy and followed by adoptive TIL transfer and IL-2. Many patients had bulky disease and had previously failed other therapies.[20]

THE FUTURE OF CANCER IMMUNOTHERAPY

The field of tumor immunology continues to expand rapidly. Advances in our understanding of the complex relationship between cancer and the immune system explain both the success and the failure of cancer immunotherapy. Much work is now focused on ways to simultaneously minimize the immunosuppressive aspects of the tumor microenvironment while maximally stimulating an antitumor immune response. Specifically, the blockade of immunosuppressive cytokines such as TGF-β, IL-10, and IL-13 may dramatically improve cell-mediated immunity to tumors. Blockade of CTLA-4, a negative costimulatory molecule, and dampening of Treg function can alleviate tumor-induced immunosuppression. In combination with immune stimulation through cancer vaccines, adoptive cell transfer, and nonspecific immunotherapy, it may be possible to achieve a balance between an effective antitumor immune response and the development of pathologic autoimmunity.

The trend for new cancer drug development is toward combination therapy. This is occurring in two ways: (1) through the design of single drugs that simultaneously target multiple pathways of tumor cell survival or (2) through the combination of novel therapies, such as immunotherapy and gene therapy, with traditional forms of treatment such as surgery, radiation therapy, and cytotoxic chemotherapy. These approaches open a new world of possibilities and challenges in cancer therapy that will have a significant impact on the way we measure efficacy and design clinical trials.

Finally, one of the most important lessons learned from cancer research since the 1990s is that what works in a mouse will not necessarily reflect the outcome in human cancer patients. Because of this, the veterinary profession is in a unique and critically important position to contribute to the success of cancer therapy through work with companion animals as translational models. In general, dogs and cats are more likely to predict treatment response and toxicity in people than are mouse models. Despite the hurdles that lie ahead, the reward will be worth the effort as the lives of people and animals with cancer are improved through a better understanding of the immune system and its enormous potential in the immunotherapy of cancer.

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Molecular/Targeted Therapy of Cancer

SECTION A
Gene Therapy for Cancer

David J. Argyle

Recombinant deoxyribonucleic acid (DNA) technology has been vigorously applied to the advancement of medicine. New molecular techniques have been exploited for the study of the role of specific genes and their products in disease, to improve diagnosis, and to produce novel therapeutics. Gene therapy, in its simplest definition, is the introduction of genes into cells *in vivo* to treat a disease.[1] Although this is one of the newest areas of medicine, the actual concept of gene therapy is not a new idea. In the late 1960s, many working in the field of molecular biology hypothesized the use of gene therapy, particularly for delivering a normal copy of a gene in a patient who had a single gene defect, such as occurs with hemophilia. However, the technology to manipulate genes and to deliver them safely to patients was not available until very recently. Even now, despite improvements in gene manipulation and delivery, many technical hurdles remain that must be overcome before gene therapy becomes accepted clinical practice.[1,2]

EFFICIENT GENE DELIVERY: THE MAJOR HURDLE TO ACHIEVING CLINICAL BENEFITS

In simple terms, gene therapy is the introduction of nucleic acid into a cell to ameliorate a disease process. For this to be effective, the gene must be delivered to a sufficient number of target cells in the body, which requires an efficient vehicle or vector for delivery. In addition, the gene must be expressed at a sufficient level and for a duration appropriate for the disease.[2]

Early studies showed how viruses were able to cause tumors by delivering their own DNA to foreign cells; this made them ideal candidates for *vectors* for gene therapy (i.e., the vehicles by which therapeutic genes could be transferred to patients). However, not until the 1980s did work on the retroviral life cycle start to revolutionize the development of gene therapy. The early retroviral studies demonstrated that retroviruses could transfer DNA to cells and that this DNA could be stably integrated into the host cell's genome. Since these early experiments, a number of viruses have been manipulated to act as vehicles for gene delivery. In addition, because of concerns about the safety of using viral vectors for gene delivery, a number of workers have explored the possibility of using *naked DNA* (a therapeutic gene delivered in a bacterial plasmid or lipid/DNA complex), with some success. Table 14-1 presents examples of the vehicles used for gene delivery.

The ideal vector would efficiently deliver the gene of interest (transgene) specifically to the cancer cell. It would be easy and cheap to manufacture, nonimmunogenic, and safe. The ideal vector does not yet exist, but great advances have been made in both viral and nonviral delivery systems.[2]

Viral Vectors

The great advantage of using viral vectors for gene delivery is the viruses' ability to infect cells and researchers' ability to exploit their replicative machinery. Most systems use replicative defective viruses to address concerns that recombination within the host may lead to the production of a wild-type virus with pathogenic potential. The common systems rely on oncogenic retroviruses (e.g., MuLV) or adenoviruses (e.g., human AD5). However, great strides are being made with lentiviral vectors (particularly HIV-1).[3-8] Most of the systems described involve local delivery of virus to tumor deposits (e.g., by intratumoral injection). Systemic delivery is hindered by rapid clearance of viruses from the body by the immune and complement systems. Work is in progress to explore cellular delivery of viruses by the systemic route. In this approach, viral producer cells are delivered to the patient, and virus production is triggered when the cells reach the tumor. Endothelial cell cultures lend themselves well to this technology, because they specifically home to areas of

TABLE 14-1	Gene Therapy Vector Systems
Vectors	**Comments**
Viral vectors	
Retroviruses (oncoviruses)	• These viruses originally were the gold standard vector. • The gene is packaged into replication-defective viral particles using a packaging cell line. • The therapeutic gene is integrated into the host cell genome when the virus is delivered to the target cell. • Oncoviruses are limited by their inability to infect postmitotic cells.
Retroviruses (lentiviruses)	• Many of these vectors are based on HIV-1. • Lentiviruses have become safer in recent years and have many of the benefits of the oncoviruses. • Lentiviruses can infect postmitotic cells. • The vector is constructed in a packaging cell line, and the therapeutic gene is integrated into the host cell genome.
Adenoviruses	• These viruses have become the most popular viral delivery mechanism. • The gene is packaged into a replication-incompetent adenovirus (usually E1 deleted). • Gene expression remains episomal when delivered to the host cell. • Concerns have been raised about the safety of adenoviral vectors, particularly possible toxic side effects at high doses. • Adenoviruses can infect a wide range of premitotic and postmitotic cells. • Conditionally replicating adenoviruses are being explored as oncolytic vectors.
Adeno-associated viruses (AAVs)	• AAVs are gaining popularity as a vector, because they are potentially safer than adenoviruses. • AAVs infect a wide variety of mammalian cell types but are limited by the amount of DNA they can deliver. • Gene expression in the host cell is episomal, but integration is possible in the natural host.
Nonviral vectors	
Naked DNA	• This is a simple form of gene delivery in which "naked" plasmid DNA is injected directly into the tumor. • Vectors are derived from bacterial plasmids and are engineered to express the therapeutic gene under the control of a strong promoter. • Naked DNA can be taken up by many tissues, but typically the efficiency for delivery is lower than that for viral gene delivery.
Particle bombardment (gene gun)	• This is a more sophisticated approach to the delivery of naked DNA. • Plasmid DNA typically is adsorbed onto gold particles. • Helium is used as a motive force to fire the gold particles into cells or tissues via a handheld "gene gun."
Liposome/DNA conjugates	• Naked DNA is surrounded with liposome to improve uptake through endocytosis. • This process enhances the efficiency of gene delivery.
Ligand/DNA conjugates	• Ligands are used to target DNA specifically to tumor tissue.

neoangiogenesis. T cells, macrophages, and dendritic cells also are being explored as potential cell delivery systems. The advantage of this system is that virus could be delivered to metastatic disease as well as primary tumors.[9-13]

Progress has been made in the development of replication-competent viruses that conditionally replicate in cancer cells. For example, the Onyx 015 vector is an E1b-deleted adenovirus that conditionally replicates in cells with a nonfunctional p53 gene.[14] p53 protein has

the potential to shut down cell cycling when infected with wild-type adenovirus but is prevented from doing so through the actions of the product of viral E1b. E1b-deficient viruses cannot replicate in normal cells with p53 intact. However, in cells that have no functional p53 protein, viral replication can proceed and cause cell lysis (Figure 14-1). Many other conditionally replicating viruses are being developed that rely on specific cancer cell defects (e.g., Reo viruses that conditionally replicate in cells with intact Ras signaling pathways) or that are transcriptionally targeted.[15-19] In one study, researchers used the osteocalcin promoter to restrict the replication of a canine adenovirus to dog osteosarcoma cells.[20] This has shown promise in preclinical evaluations and has been shown to produce a therapeutic benefit *in vivo*. However, most dogs in the United States and Europe are vaccinated against canine adenovirus, and these vectors may not be able to overcome host immunity.

Nonviral Gene Delivery

Concerns about virus safety and the inability to produce high enough viral titers have led to the development of nonviral delivery systems for gene therapy.[21] Liposomes have been used to deliver genes to tumor cells safely and efficiently through direct injection.[22,23] Naked DNA

Normal cells (Intact p53)

→ No effect

E1b deleted Ad5

→ Viral oncolysis

Tumor cells (Mutated p53)

Figure 14-1

Conditionally replicating adenovirus. The Onyx 015 vector is an E1b-deleted adenovirus that conditionally replicates in cells with a nonfunctional p53 gene. p53 protein has the potential to shut down cell cycling when infected with wild-type adenovirus but is prevented from doing so through the actions of the product of viral E1b. E1b-deficient viruses cannot replicate in normal cells with p53 intact. However, in cells that have no functional p53 protein, viral replication can proceed and cause cell lysis.

(i.e., plasmid DNA alone containing the gene of interest) has been shown to be taken up by tumor cells and antigen-presenting cells after simple direct injection. Particle-mediated gene delivery using a "gene gun" is a modification of this approach. DNA is adsorbed onto gold particles and fired into tissues under high pressure (using helium as the motive force).[24,25] However, most of these techniques still are inefficient vehicles for delivery and cannot be used systemically (see Table 14-1).

TARGETED GENE DELIVERY

A major barrier to widespread clinical use of gene therapy is the inability to give vectors systemically and to ensure that therapeutic transgenes are not expressed in normal cells. Numerous strategies have been attempted to accomplish targeting and spare normal tissue. Previously, the use of conditionally replicating viruses was described, which is one method of targeting. Surface modification of viruses (transductional targeting) also is being explored. An example of this is the use of modified fibers on the surface of adenoviruses that allow the virus to enter only cells with specific receptors.

Another strategy is transcriptional targeting once the vector has entered the cell.[26-33] Although every gene is represented in every cell of the body, expression of any one gene requires specific transcription factors that may be unique to a particular cell or tissue type. Certain genes have been identified that are expressed in cancer cells but not in normal cells (e.g., telomerase) or are expressed only in a specific tissue type (e.g., prostate-specific antigen [PSA]). By using the promoter sequences for these genes to drive transgene expression, targeted expression in cancer cells only (e.g., using the promoter for telomerase) or in a specific tissue type (e.g., in the prostate using the promoter for PSA) can be achieved.
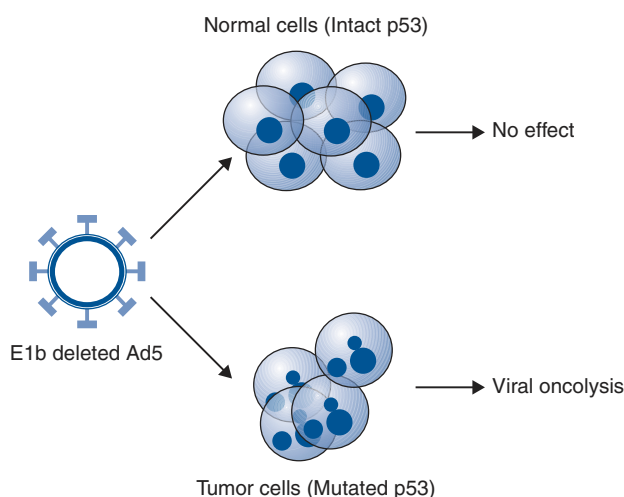
GENE THERAPY STRATEGIES FOR CANCER

Despite advances in surgical techniques and the use of radiotherapy and chemotherapy, cancer remains a disease of high mortality in both human and veterinary medicine, warranting the investigation of alternative treatments. Gene therapy has the potential to play a major role in the development of new cancer therapeutic agents. Four broad approaches can be applied: rescue of the cancer cell through gene replacement technology, destruction of cancer cells through delivery of "suicide genes," gene-directed immunotherapy, and delivery of chemoprotective genes.

Rescue of the Cancer Cell through Gene Replacement Technology

A better understanding of the molecular events in cancer has made possible the identification of defective genes involved in the cancer phenotype. One of the most studied genes in cancer development has been the tumor suppressor gene p53. p53 acts as a genomic guardian for the cell; it is switched on when a cell's DNA is damaged. The product of this gene causes the cell either to stop dividing or to become apoptotic (programmed cell death), depending on the degree of damage. In many cancers this gene is defective; damaged cells fail to stop dividing and can accumulate further damaging events, which can allow selection for a malignant phenotype. A number of studies have addressed this by attempting to replace the defective p53 gene with its normal counterpart.[34] However, problems arise with this approach: (1) the current technology is incapable of delivering a normal p53 gene efficiently to every cancer cell in a tumor mass; and (2) cancer is a multigenetic abnormality, and the delivery of one correct gene to a tumor cell still may not have the desired phenotypic effect.

A more promising approach has been to use the lack of a normal functioning p53 gene to target viruses to kill cells. Use of E1b-deleted adenoviruses specifically to cause oncolysis in p53 null cells has proved successful in some human clinical models.[20] p53 mutations in domestic species such as the dog and cat also have been well characterized and may provide targets for therapy, particularly in diseases such as canine osteosarcoma and feline vaccine-associated sarcomas.

Destruction of Cancer Cells through Delivery of "Suicide Genes"

Typically the "suicide gene" approach involves the delivery to cancer cells of a gene (usually an enzyme) that can convert a relatively nontoxic prodrug into a compound that can kill the cancer cell (gene-directed enzyme prodrug therapy [GDEPT]). In a clinical application, the gene would be delivered to the tumor, and the enzyme activity would be confined to the cancer cells.[35] The patient then would be given a prodrug systemically. In the cancer cells, the novel enzyme would convert the prodrug to a more active compound that has the ability to kill the cancer cell (Figure 14-2). A number of successful systems have been developed based on this system. For example, the *Escherichia coli* nitroreductase gene has been used in preclinical models to cause reduction of an inactive prodrug (CB1954, a weak alkylating agent) to promote cell killing in cancer cells.[35] However, because of the low efficiency of existing vectors, the success of this therapy largely will depend on the extent of the *bystander effect*. In this effect, activation of the prodrug in the cell causes cell death and also leakage of toxic metabolites to neighboring cells. Consequently, it is estimated that only a small fraction of the cells need to receive the gene to produce a dramatic effect on the tumor as a whole. Furthermore, in mouse models a distant bystander effect on tumor metastases has been demonstrated that is mediated through the patient's immune system.[29] The *in situ* destruction of tumor cells is mediated through necrosis rather than apoptosis, creating an ideal inflammatory environment for the exposure and presentation of tumor antigens to the immune system. This allows the patient's immune system to recognize tumor metastases and has caused regression in a number of preclinical model systems. These systems have been combined with transcriptionally targeted vectors (described previously) to improve the eventual therapeutic index.

Gene-Directed Immunotherapy

The search for an effective cancer vaccine over the past 150 years has led to extensive studies of the immune response of cancer patients. These studies have suggested that cell-mediated immune responses are important components of the antitumor immune response. Cytokines are small glycoprotein molecules that orchestrate the immune response, tissue repair, and hemopoiesis, and it has been demonstrated that the relative amounts of individual cytokines can direct the immune system toward either a mainly humoral or a mainly cell-mediated response. In particular, cytokines such as interleukin-2 (IL-2), interferon gamma (IF-γ), IL-12, and IL-18 have the ability to promote cell-mediated responses. Furthermore, evidence derived from animal models suggests that local production of cytokines around a tumor mass can lead to production of an antitumor immune response and reversal of T-cell anergy (unresponsiveness).[36,37] Therefore there appears to be a rationale for using cytokine molecules in cancer patients to improve the immune response to tumors that present weakly antigenic epitopes or epitopes that evade immune recognition.

In the 1980s and 1990s a number of clinical studies were undertaken using recombinant cytokine proteins to improve the survival of human cancer patients. However, cytokines tend to be autocrine or paracrine in nature, and the levels of protein required to demonstrate a biologic effect often were too toxic for the patient to withstand. A more promising approach has been to deliver the actual cytokine genes to cancer cells rather than delivery of the protein to the whole patient.[38-40] This approach also has been used in a number of small-scale veterinary studies, including one involving canine malignant melanoma in which cells were used to deliver IL-2 to tumors.[41] These studies have
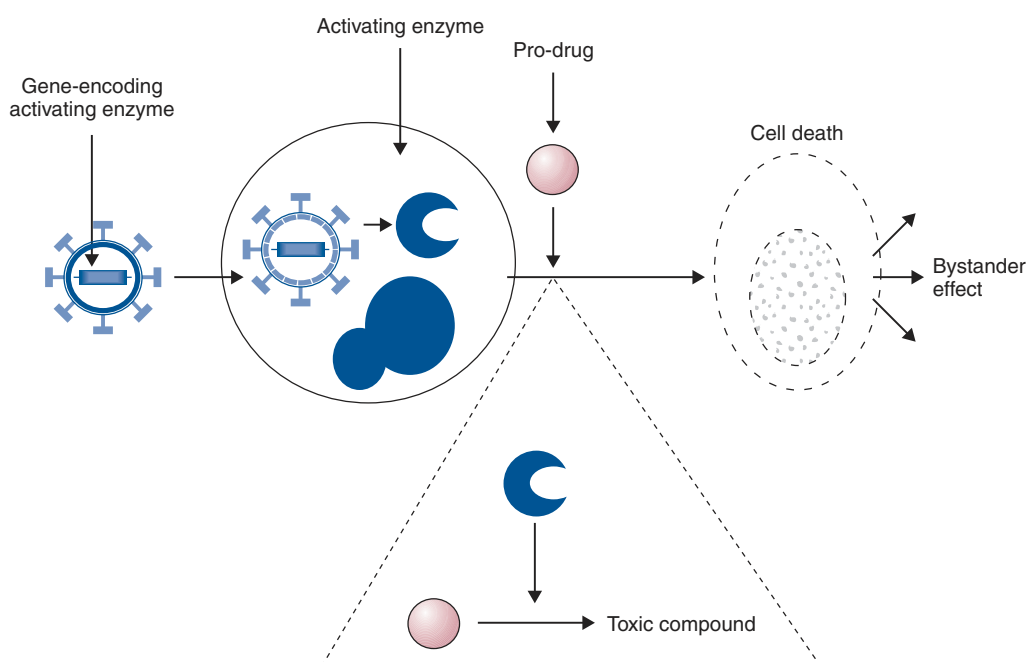
Figure 14-2

Gene-directed enzyme prodrug therapy (GDEPT). With GDEPT, an activating gene is delivered to the cancer cells. A relatively inactive prodrug is then given to the patient systemically. In cells that process the activating gene, the prodrug is converted to a highly toxic drug that can kill the cancer cell. The advantage of this system is the evidence of bystander effect; that is, only a small proportion of cancer cells need to receive the activating gene, because toxic metabolites leak across gap junctions and kill surrounding cancer cells.

produced encouraging results, which warrant larger scale trials.

As explained previously, *in situ* destruction of tumor cells by enzymes can lead to a distant bystander effect mediated by the immune system. Many trials have combined this technique with a gene-directed immunotherapy approach. Cytokine genes and the prodrug-activating gene are delivered to cancer cells. The conversion of the inactive prodrug by the activating gene leads to destruction of the cancer cells by means of necrosis. The codelivery of cytokines that enhance cell-mediated immune responses (e.g., IL-2, IF-γ, IL-12, and IL-18) enhances the antitumor response and may improve the distant bystander effect against micrometastatic disease.[1,2]

Delivery of Chemoprotective Genes

An alternative approach to gene therapy for cancer, this method involves the delivery of genes to normal cells of the bone marrow to protect them against the cytotoxic effects of conventional chemotherapeutic drugs. In particular, the multidrug resistance (MDR) gene has been cloned and delivered to normal bone marrow cells.

When patients are given high doses of chemotherapy, the normal cells with the MDR gene are able to export the toxic drugs across their membranes and reduce potential side effects.[42-44] However, this approach does not protect gastrointestinal cells, which will still be a limiting effect of this technique. There is also a danger that the MDR gene could be transferred to malignant cells, rendering them insensitive to the effects of standard drugs.

SAFETY CONSIDERATIONS IN GENE THERAPY

Safety is a primary consideration in gene therapy, particularly the safety of the vectors used for gene delivery. Until recently many gene therapy trials had used retroviral vectors, which offer many advantages (see Table 14-1). However, retroviruses are also associated with serious diseases of domestic animals, and their use in gene therapy poses a risk of *insertional mutagenesis*, or the production of replication-competent viruses during the manufacturing process. In real terms, insertional mutagenesis is unlikely to lead to a malignant transformation, because cancer is a

multistep process. In fact, the risk of malignant transformation may be greater with external beam radiation than with the use of retroviruses. The production of replication-competent retroviruses during the manufacturing process also is unlikely because of the rigorous testing required before clinical application.

In recent years a shift has occurred in gene therapy from the use of retroviruses to the use of viruses such as adenoviruses and the human adeno-associated viruses (AAVs). Adenoviruses may also pose some risks, including inappropriate inflammatory responses that lead to serious clinical complications. However, a great deal of work currently is underway to improve viral vectors by removing most of their genetic material to produce "ghost vectors." These potentially would be less toxic and offer a brighter future for virally mediated gene transfer.

The delivery of naked DNA may seem to offer a safer alternative. However, all the potential safety issues with this technology have not yet been fully resolved. These issues include the possible risks of autoimmunity and the actual fate of the DNA after its delivery to the patient. In the case of autoimmunity, however, preclinical models have shown no evidence of it being a problem.[45]

NEW HORIZONS

Gene therapy promises a novel approach to the treatment of cancer and represents the newest area of pharmacology. A number of technical issues, such as the safety aspects of delivery and the efficiency of the vectors, must be resolved before gene therapy becomes established clinical practice. Although gene therapy is very much in its infancy, advances in the field are proceeding at a rapid pace. A number of clinical trials have begun in companion animals, and products are in development for clinical application. However, although these treatments appear to be powerful in preclinical models, they are likely to have the greatest benefit in the management of patients with minimal disease. In such cases gene therapy probably will be best used not as a stand-alone treatment but as an adjunct to more conventional therapies such as surgery, radiation, or chemotherapy.

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SECTION B
Signal Transduction and Cancer

Cheryl London

In normal cells, signals are generated that begin at the outside of the cell and transmit through the cytoplasm to the nucleus, regulating cell growth, differentiation, survival, and death. Over the past few years, researchers have recognized that many of the components of the signal transduction pathways are dysregulated in cancer cells, leading to uncontrolled cell growth and thereby contributing to tumorigenesis. Because many tumors have similar alterations in signal transduction components, these components have become a promising target for therapeutic intervention. This section focuses on the role of a particular group of signal transducers, called *protein kinases*, their role in normal cells, the mechanisms by which they contribute to tumorigenesis, and the development and application of agents designed to inhibit them when they become dysfunctional.

PROTEIN KINASES AND NORMAL CELLS

Protein kinases play critical roles in normal cell signal transduction, acting to tightly regulate a variety of critical cellular processes such as growth and differentiation. These proteins work through *phosphorylation*; that is, they bind adenosine triphosphate (ATP) and use it to add phosphate groups to key residues on themselves (a process called *autophosphorylation*) and on other molecules, resulting in a downstream signal inside the cell. This process usually occurs in response to external signals generated by growth factors or other stimuli that initiate the cascade. Protein kinases are divided into two groups: tyrosine kinases (TKs), which phosphorylate proteins on tyrosine residues and serine/threonine kinases, which phosphorylate proteins on serine and/or threonine residues. In some cases the kinases perform both functions (i.e., dual function kinases). The kinases generally are divided into those expressed on the cell surface, those in the cytoplasm that act as a bridge, and those in the nucleus. The human genome encodes approximately 518 kinases, of which 90 are classified as tyrosine kinases.[1]

Tyrosine kinases on the cell surface that are activated through binding of growth factors are called *receptor tyrosine kinases (RTKs)*. Of the 90 identified tyrosine kinases, 58 are known to be RTKs. Each RTK contains an extracellular domain that binds a growth factor, a transmembrane domain, and a cytoplasmic kinase domain that positively and negatively regulates phosphorylation of the RTK (Figure 14-3).[2,3] The RTKs are grouped into

families based on their overall structure. For example, the split kinases (i.e., fibroblast growth factor receptor [FGFR], vascular endothelial growth factor receptor [VEGFR], and platelet-derived growth factor receptor [PDGFR]) have a cytoplasmic domain in which the ATP binding pocket and catalytic domain are separated by a kinase insert (Figure 14-3). Most of the RTKs are monomers on the cell surface and are dimerized through binding of a growth factor; this changes the three dimensional structure of the receptor, permitting ATP to bind and autophosphorylation to occur, resulting in generation of a downstream signal through subsequent binding of adaptor proteins and nonreceptor kinases. Other examples of RTKs are Kit, Met, and epidermal growth factor receptor (EGFR), all of which have been demonstrated to play prominent roles in particular cancers.[4-7]

Normal kinase function, although clearly critical to the regulation of cell functions, also has been shown to be important in the process of new blood vessel growth into tumors *(angiogenesis)*, because most tumors do not grow beyond a few millimeters unless they create their own blood supply. The RTKs known to be crucial to this process are VEGFR, PDGFR, FGFR, and Tie-1 and Tie-2 (receptors for angiopoietin).[8-11] VEGFRs are expressed on vascular endothelium and VEGFR signaling is critical for endothelial migration and proliferation.[8] PDGFR is expressed in stroma and pericytes, and PDGF has been demonstrated to cause endothelial cells to proliferate and migrate.[10,11] FGFR is expressed on vascular

endothelium and works with VEGFR to promote increased expression of VEGF.[10] Tie-1 and Tie-2 are expressed on blood vessels in tumors and are important in the recruitment of pericytes and smooth muscle cells to the newly forming vascular channels.[12]

Kinases in the cytoplasm act as a bridge, conducting signals generated by the receptor kinases on the cell surface to the nucleus through a series of intermediates that become phosphorylated.[13] The cytoplasmic kinases may be directly on the inside of the cell membrane or free in the cytoplasm itself. With respect to tumor cell biology, two particular cytoplasmic pathways are known to be dysregulated in a number of cancers. The first pathway includes members of the RAS-RAF-MEK-ERK/p38/JNK families (Figure 14-4).[14,15] Most of these are serine/threonine kinases linked to signals generated by RTKs. Their activation leads to ERK phosphorylation and subsequent translocation into the nucleus, which ultimately alters the activity of transcription factors and nuclear kinases important for controlling the cell cycle. In human cancers a number of these kinases have been found to be dysregulated. Examples include RAS mutations commonly found in lung cancer, colon cancer, and several hematologic malignancies and BRAF mutations found in cutaneous human melanomas and papillary thyroid carcinomas.[16-18]

The second cytoplasmic pathway includes phosphatidyl inositol-3 kinase (PI3K) and its associated downstream signal transducers AKT, NFkB, and mTOR, among others (Figure 14-5).[19,20] PI3K is activated by

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Figure 14-3

Structure of receptor tyrosine kinases (RTKs). The structure of RTK families implicated in a variety of malignancies is shown. *EGFR*, Epidermal growth factor receptor; *FGFR*, fibroblast growth factor receptor; *PDGFR*, platelet-derived growth factor receptor; *VEGFR*, vascular endothelial growth factor receptor; *IGF-1R*, insulin-like growth factor receptor 1; *TRK*, member of a nerve growth factor receptor family; *Eph*, member of an ephrin receptor family; *Tie*, tyrosine kinase receptor on endothelial cells; *ALK*, anaplastic lymphoma kinase; *AB*, acid box; *CadhD*, cadherin-like domain; *CRD*, cysteine-rich domain; *EGFD*, epidermal growth factor–like domain; *IgD*, immunoglobulin-like domain, *LRD*, leucine-rich domain. The symbols α and β indicate specific RTK subunits. *(From Vet Comp Oncol 2:177-193, 2004.)*

Figure 14-4

RAS signal transduction. Activated receptor tyrosine kinases recruit SOS to the plasma membrane through binding of SHC and GRB-2. SOS replaces bound GDP with GTP, thereby activating RAS. The downstream target, RAF, is phosphorylated by RAS, leading to subsequent activation of MEK and then ERK. ERK has several substrates in both the nucleus and the cytoplasm, including ETS transcription factors such as ELK-1 and RSK, which regulate cell cycle progression. *(From Vet Comp Oncol 2:177-193, 2004.)*

RTKs after their autophosphorylation. It then activates AKT, which alters several additional proteins involved in the regulation of cell survival, cycling, and growth.[21] AKT phosphorylates targets that promote apoptosis (BAD, procaspase-9, and Forkhead transcription factors) and activates NFkB, a transcription factor that has antiapoptotic activity.[19-21] AKT also phosphorylates other proteins such as mTOR, p21, p27, and GSK3. This leads to redistribution of these proteins either into or out of the nucleus, ultimately inhibiting apoptosis while promoting cell cycling.[19-21] Both PI3K and AKT have been found to be overexpressed in several human cancers, including those of the cervix, ovaries, pancreas, and breast.[22-25] This pathway may also become dysregulated through loss of activity of a phosphatase called PTEN, which normally acts to dephosphorylate AKT.[19,26,27] PTEN mutations are found in a variety of human cancers (e.g., glioblastoma and prostate cancer)

and result in excessive AKT activity, which promotes malignant transformation.[26]

After a signal has been conducted from the cell surface through the cytoplasm, it enters the nucleus. This alters both gene transcription and the proteins that control cell cycling. The cyclins and their kinase partners (cyclin-dependent kinases [CDKs]) act to regulate the progression of cells through various phases of the cell cycle (Figure 14-6).[28-30] The cyclins comprise several families. Cyclins D and E control restriction point passage by activating their respective CDKs (CDK4 and CDK6 for cyclin D and CDK2 for cyclin E). Coordinated function of cyclins D and E is required for cells to progress from G_1 into S phase (Figure 14-6). In many cases, RTK-generated signals induce expression of cyclin D, which complexes with CDK4 and CDK6, resulting in phosphorylation of the tumor suppressor Rb, partially repressing its function.[29,30] Functional cyclin D/CDK

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Figure 14-5

Phosphatidyl inositol-3 kinase (PI3K) signal transduction. After receptor tyrosine kinase activation, PI3K is recruited to the phosphorylated receptor through binding of the p85 adaptor subunit, leading to activation of the catalytic subunit (p110). This activation results in the generation of the second messenger phosphatidyl inositol-3,4,5-triphosphate (PIP3). PIP3 recruits AKT to the membrane and, after its phosphorylation, several downstream targets subsequently are phosphorylated, leading to either their activation or inhibition. The cumulative effect results in cell survival, growth, and proliferation. *(From Vet Comp Oncol 2:177-193, 2004.)*

complexes induce transcription of cyclin E, and active cyclin E/CDK complexes further reduce Rb activity through phosphorylation. This in turn initiates the process of DNA replication important for cell cycling. Overexpression of cyclins D and E is found in human cancers, including those of the breast, pancreas, head, and neck.[30]

PROTEIN KINASES AND CANCER CELLS

Over the past several years, dysfunction of several protein kinases has been characterized in a variety of human cancers. It is just beginning to be investigated in spontaneous tumors of dogs and cats (Table 14-2). Kinases may be dysregulated through a variety of mechanisms, including mutation, overexpression, fusion proteins, or autocrine loops. In the case of mutations, these may result in phosphorylation of the kinase in the absence of an appropriate signal. Such mutations can consist of a single amino acid change through a point mutation, deletion of amino acids, or insertion of amino acids, usually in the form of an internal tandem duplication (ITD). An example of a point mutation occurs in the BRAF gene in approximately 60% of human cutaneous melanomas.[16,31,32] In most instances, the mutation induces a specific amino acid change (V599E, exon 15), which causes a conformation change in the protein mimicking its activated form (i.e., inducing constitutive activation), thereby resulting in constitutive ERK signaling and abnormal promotion of cell growth and survival.[33,34] RAS is another kinase that is dysregulated through point mutation in several hematopoietic neoplasms (multiple myeloma, juvenile chronic myelogenous leukemia [CML], acute myelogenous leukemia [AML], and chronic myelomonocytic leukemia [CMML])

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Figure 14-6

Cyclin and cyclin-dependent kinase (CDK) regulation of G1-S transition. CDK inhibitors (e.g., p16ink4 and p21) restrict the activity of cyclin D– and cyclin E–dependent kinases. Progressive Rb phosphorylation by the cyclins results in liberation of E2F and the resultant transcription of S phase genes. *(From Vet Comp Oncol 2:177-193, 2004.)*

and in lung cancer, colon cancer, and many others.[15,35,36]

Another example of a mutation involves Kit, an RTK that normally is expressed on hematopoietic stem cells, melanocytes, in the central nervous system, and on mast cells.[37] In approximately 25% to 30% of canine grade II and grade III mast cell tumors, mutations consisting of ITDs are found in the juxtamembrane domain of Kit. These mutations result in constitutive activation of Kit in the absence of ligand binding and are associated with a higher risk of local recurrence and metastasis.[38-40] Kit mutations consisting of deletions in the juxtamembrane domain are also found in approximately 50% of human patients with gastrointestinal stromal tumors (GISTs).[41-47] Another example involves human acute myelogenous leukemia, in which ITDs in

the RTK Flt3 are found in 30% of affected patients.[48-51] Similar to the case with Kit, these mutations occur in the juxtamembrane domain, resulting in constitutive activation of the protein. Such mutations have been linked to an increased risk of relapse and a poor overall survival rate.

Overexpression of kinases usually involves the RTKs and may result in enhanced response of the cancer cells to normal levels of growth factor; or, if the levels are high enough, the kinase may become activated through spontaneous dimerization in the absence of signal/growth factor. In humans, the RTK HER2 (also known as ErbB2, a member of the epidermal growth factor family) is overexpressed in both breast and ovarian carcinomas.[2,52,53] Such overexpression of HER2 correlates with a worse outcome in human breast cancer patients, because these tumors often behave in a more aggressive manner.[52] EGFR, a related RTK, also is overexpressed in human lung, bladder, cervical, ovarian, renal, and pancreatic cancers, and some tumors have as many as 60 copies of the gene per cell.[5,54,55] As with HER2, such overexpression is linked to a worse outcome in affected patients.[5]

Fusion proteins are generated when a portion of the kinase becomes attached to another gene through chromosomal rearrangement and the normal mechanisms that control protein function are disrupted. Examples include TEL-PDGFRβ in CMML, FIP1-PDGFRα in hypereosinophilic syndrome with mastocytosis, and the Bcr-Abl fusion protein, which is found in 90% of patients with CML.[56-59] Abl is a cytoplasmic tyrosine kinase that, when fused to Bcr, results in dysregulation of Abl, inappropriate activity of the protein, and resultant malignant transformation.

Autocrine loops of activation primarily occur when the tumor cell expresses both the RTK and the growth factor; in most cases, one or the other usually is also overexpressed, resulting in constitutive activation of the RTK. Examples include coexpression of TGFα and EGFR in glioblastoma and squamous cell carcinoma, insulin-like growth factor (IGF) and its ligand, IGF-1R, in breast and colorectal cancer, and VEGF and VEGFR in melanoma.[2,60-62] In canine cancers, autocrine loops have been documented in osteosarcoma (OSA) (coexpression of Met and its ligand, HGF) and hemangiosarcoma (HSA) (coexpression of Kit and its ligand, SCF).[63-65]

INHIBITION OF KINASES

Given the detailed characterization of dysregulation of signal transducers in cancer cells, a significant effort has been directed at developing strategies to inhibit kinases that participate in tumorigenesis at the level of both the cancer cell and the endothelial cell in an effort to inhibit angiogenesis. The two most successful

TABLE 14-2   Receptor Tyrosine Kinases Associated with Cancer

Tyrosine Kinase	Cancer Association
EGFR family	Breast, ovary, lung, stomach, and colon cancer; glioblastoma
Insulin receptor family	Sarcomas; cervical, kidney cancer
PDGFR family	Glioblastoma, ovarian cancer, chronic myelomonocytic leukemia (CMML)
Kit	AML, gastrointestinal stromal tumor (GIST), seminoma, mastocytosis/mast cell tumor, lung cancer
Flt3	AML
VEGFR family	Angiogenesis, Kaposi's sarcoma, hemangiosarcoma, melanoma
FGFR family	AML; lymphoma; breast, prostate cancer; multiple myeloma
NGFR family	Papillary thyroid cancer, neuroblastoma, fibrosarcoma, AML
Met/Ron	Papillary thyroid cancer; osteosarcoma; rhabdomyosarcoma; liver, kidney, colon cancer
EPHR family	Melanoma; stomach, colon, breast, esophageal cancer
AXL	AML
Tie family	Angiogenesis, stomach cancer, hemangioblastoma
RET family	Thyroid cancer, multiple endocrine neoplasia
ALK	Non-Hodgkin's lymphoma

EGFR, Epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; NGFR, nerve growth factor receptor.

approaches have been antireceptor antibodies and small molecule kinase inhibitors.

Several antibodies have been developed to target the extracellular domain of RTKs. These antibodies may prevent the growth factor from binding or may induce an immune response against the cancer cell. The most successful example of this approach is a humanized monoclonal antibody called trastuzumab (Herceptin; Genentech). This antibody targets HER2, an EGFR family member that is overexpressed in approximately 30% of breast cancers and also in some other cancers, including prostate cancer, ovarian cancer, and non-small-cell lung cancer (NSCLC).[66] In clinical trials, Herceptin has demonstrated significant activity against HER2-positive breast cancer, resulting in response rates of approximately 25% in patients with metastatic disease.[67] When Herceptin was combined with chemotherapy (paclitaxel or doxorubicin), the response rate approached 50%.[68] Another antibody, cetuximab (Erbitux; Imclone), targets the ErbB1/HER1 EGFR, which is known to be overexpressed by several carcinomas.[5,66]

Small molecule inhibitors work by blocking the ATP binding site of kinases, essentially acting as competitive inhibitors. In the absence of ATP, the kinase is unable to phosphorylate itself or downstream signaling elements. To allow the development of inhibitors specific for particular kinases, the ATP binding pockets of many receptors have been characterized, and this has been used to construct competitive inhibitors that bind only the pocket of a particular kinase family (e.g., the FGFRs). Such inhibitors often are easy to synthesize in large quantities, orally bioavailable, and can readily enter cells to gain access to the intended target.

Perhaps the most successful small molecule kinase inhibitor is imatinib (Gleevec; Novartis), an orally administered drug that binds the ATP pocket of Abl. As previously discussed, approximately 90% of human patients with CML have a fusion protein of the cytoplasmic Abl with Bcr (Bcr-Abl) that results in uncontrolled Abl activation. Several clinical trials of Gleevec have been conducted in individuals with CML.[69-74] In patients in the chronic phase of the disease, Gleevec induces a remission rate of close to 95%, and most patients remain in remission for longer than 1 year. Unfortunately, the remission rate is much lower for patients in blast crisis (20% to 50%); in these individuals, remission lasts, on average, less than 10 months. Resistance to Gleevec often is due both to Bcr-Abl gene amplification and to mutations in the ATP binding pocket that prevent appropriate binding of the inhibitor.[75,76]

Gleevec also was found to block the ATP binding site of another kinase, the RTK Kit. As mentioned previously, Kit dysregulation is common in many human cancers and also has been identified in canine cancers. One such example is GIST, in which 60% to 90% of the tumors have point mutations or deletions in the juxtamembrane domain of Kit, resulting in constitutive activation.[42,77] GISTs are known to be resistant to chemotherapy, and the prognosis for affected individuals usually is very poor.[78,79] Clinical trials of Gleevec in the treatment of GIST have shown response rates of

50% to 70%, far better than the 5% response rate seen with standard chemotherapy. A small number of patients with GIST have tumors that do not have Kit mutations, but rather have activating mutations in PDGFRα; these patients also respond to Gleevec, because the drug is known also to inhibit autophosphorylation of this RTK.[80]

Another class of kinase inhibitors, developed by Pfizer, selectively inhibits the tyrosine kinase activity of several members of the split kinase RTK family, including VEGFR, PDGFR, and Kit.[81] Like Gleevec, the selective inhibitors sit in the ATP binding pocket of these receptors. Two of the selective inhibitors, SU11248 and SU11654, have demonstrated significant activity in phase I and phase II human clinical trials. Of humans with Gleevec-resistant GIST, 13% achieved a partial response to SU11248 and 65% achieved stable disease (reported at the American Society for Clinical Oncology, 2004). Of patients with renal cell carcinoma in whom IL-2 and/or interferon therapy had failed, 33% achieved a partial response and 37% experienced stable disease (reported at the American Society for Clinical Oncology, 2004).

In veterinary medicine, a phase I trial was undertaken to explore the safety and efficacy of SU11654, the veterinary counterpart to SU11248.[82] Fifty-seven dogs with a variety of spontaneous neoplasms were enrolled in the study. Measurable objective responses were observed in 16 dogs for an overall response rate of 28% (16 of 57). Stable disease for longer than 10 weeks was seen in 15 dogs, for a resultant overall biologic activity rate of 54% (31 of 57). The highest response rate was observed in mast cell tumors (MCTs), which, as previously described, often are driven by aberrant Kit signaling. This study provided the first evidence that multitargeted kinase inhibitors can exhibit broad activity against a variety of spontaneous malignancies. SU11654 currently is undergoing further clinical evaluation in dogs with cancer.

Another orally active, small molecule inhibitor that has been successful in treating human cancers is gefitinib (Iressa; Astra Zeneca). This drug acts to block EGFR signal transduction and, like Gleevec, sits in the ATP binding pocket, acting as a competitive inhibitor.[83,84] The EGFR family is an attractive target for inhibition, because many human cancers, including breast cancer, lung cancer, and bladder cancer, overexpress one or more family members.[5] In human patients, Iressa has clinical activity against NSCLC, with 12% to 20% of patients experiencing a complete or partial response, and 30% to 40% of patients experiencing stable disease.[5,83] It recently was shown that the likelihood of response to Iressa and other EGFR inhibitors correlates with the presence of a point mutation in the kinase domain of the receptor that is found primarily in patients with non-smoking-induced lung cancers (bronchoalveolar carcinomas).[85] These mutations induced prolonged signal transduction when the receptor was stimulated by EGF, resulting in an increased duration of downstream signal transduction, promoting uncontrolled growth and survival. Unfortunately, Iressa has demonstrated little activity against breast cancer, but promising results have been observed for head and neck, prostatic, and ovarian cancers when used as a single agent.[5,83,84] Another such inhibitor, erlotinib (Tarceva; OSI Pharmaceuticals), currently is in phase III trials for NSCLC and has a spectrum of activity similar to that of Iressa.

An attractive target recently identified for potential therapeutic intervention is RAF, because it is mutated in several human cancers, and it is one of the signaling intermediates in the RAS/RAF/MEK/ERK pathway, which often is dysregulated in tumor cells. BAY 43-9006 (Bayer) is derived from the bis-aryl ureas and initially was identified through a screening of thousands of medicinal chemistry compounds for activity against RAF.[86-88] In a phase II clinical trial of patients with advanced renal cell carcinoma (RCC), BAY 43-9006 induced a minimal response in 23% of patients and a partial response in 12%; 36% experienced stable disease (reported at the American Society for Clinical Oncology, 2004). Studies are underway to evaluate the efficacy of this drug against malignant cutaneous melanoma, because more than 60% of patients have tumors that have activating mutations in BRAF, which suggests that this kinase is likely to serve as a common target for therapeutic inhibition.

Flavopiridol, a partly synthetic flavonoid derived from an indigenous plant (rohitukine) found in India, sits in the ATP binding pocket of CDK2, acting as a competitive inhibitor.[89,90] This compound has been shown to inhibit most of the CDKs evaluated, although some of them less potently than others.[91,92] Although flavopiridol had encouraging activity *in vitro* and in mouse models, phase I and phase II human studies have failed to demonstrate significant efficacy when the drug was used as a single agent.[89] Moreover, toxicities, including diarrhea, nausea and vomiting, fatigue, and venous thrombosis, have hampered efforts to increase the systemic drug concentrations and thereby improve the clinical outcome. Studies combining flavopiridol with standard chemotherapy treatments are underway to determine whether better clinical responses can be achieved using this drug.

CONCLUSION

With the advent of molecular techniques, it has become increasingly easier to identify common signal transduction pathways that are dysfunctional in cancer cells. This has led to the rapid development of inhibitors

capable of blocking specific pathways that may be critical for cancer cell survival. The recent success of Gleevec and SU11654 provides evidence that such strategies can markedly improve clinical outcome. Ultimately, therapeutic efficacy will depend on careful characterization of specific signal transduction pathways in normal cells and their dysregulation in cancer cells to identify appropriate avenues for therapeutic intervention. Perhaps the greatest challenge will be determining how these novel therapeutics can be effectively combined with standard treatment regimens such as chemotherapy and radiation therapy to provide optimal anticancer efficacy.

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SECTION C
RNA Interference

David J. Argyle

During the late 1980s, scientists working on the color pigments of petunias observed a phenomenon they called posttranscriptional gene silencing (PTGS).[1] They demonstrated that silencing of an endogenous gene at the posttranscriptional level could occur through the introduction of a homologous transgene. Since these initial observations, it has been demonstrated that PTGS could occur through the introduction of homologous double-stranded ribonucleic acid (dsRNA) and viruses, as well as transgenes, in plants and some insect species.[1] The use of dsRNA to cause posttranscriptional gene silencing subsequently was referred to by the umbrella term *RNA interference* (RNAi). Eventually, researchers recognized that RNAi was a natural phenomenon in insects, protozoa, and plants, in which it was considered to be an evolutionarily conserved defense mechanism.[1]

GENE SILENCING IN MAMMALIAN CELLS

The existence of RNAi as a natural phenomenon in mammalian cells took much longer to establish. In mammalian cells, the introduction of large dsRNA molecules leads to nonspecific suppression of gene expression rather than the highly specific gene targeting observed in other species. This happens because dsRNA induces a nonspecific antiviral response in mammalian cells. In this response, dsRNA can activate RNase-L (leading to nonspecific RNA degradation) or the protein kinase PKR, which phosphorylates and inactivates the translation initiation factor, eIF2a, leading to repression of translation.[2]

In insect cells it had been observed that dsRNA is cleaved to form small interfering RNA (siRNA), which targets messenger RNA (mRNA) in a sequence-specific manner.[3] With the knowledge that dsRNA molecules shorter than 30 nucleotides could bypass the antiviral response, researchers investigated whether the introduction of siRNA oligonucleotides in mammalian cells could induce gene-specific silencing. They found that transient transfection of siRNA could cause sequence-specific RNAi in mammalian cells. Through lengthy optimization experiments, scientists discovered that the most potent silencing molecules were 21 nucleotides long with a 2-nucleotide overhang at the 3′ end. Sequence specificity is highly stringent, and even a single base mismatch between the siRNA and mRNA may dramatically reduce the silencing effect.[3]

CURRENT MODEL FOR RNA INTERFERENCE

The complete mechanism of RNAi has yet to be elucidated. However, both biochemical and genetic approaches have led to the current working model, which consists of an initiation step followed by an effector step. In the initiation step, long dsRNA molecules are digested by the enzyme Dicer, a member of the RNase III family of dsRNA-specific ribonucleases. Dicer activity leads to successive ATP-dependent cleavage events that degrade the RNA to 19 to 21 base pair (bp) duplexes (siRNA), each with 2-nucleotide 3′ overhangs (Figure 14-7).

In the effector step, the siRNA bind to a nuclease complex to form the RNA-induced silencing complex (RISC). Activation of RISC occurs through ATP-dependent unwinding of the siRNA duplex. Through base pair interactions, the RISC targets the homologous mRNA transcript and then cleaves the mRNA approximately 12 nucleotides from the 3′ terminus of the siRNA.
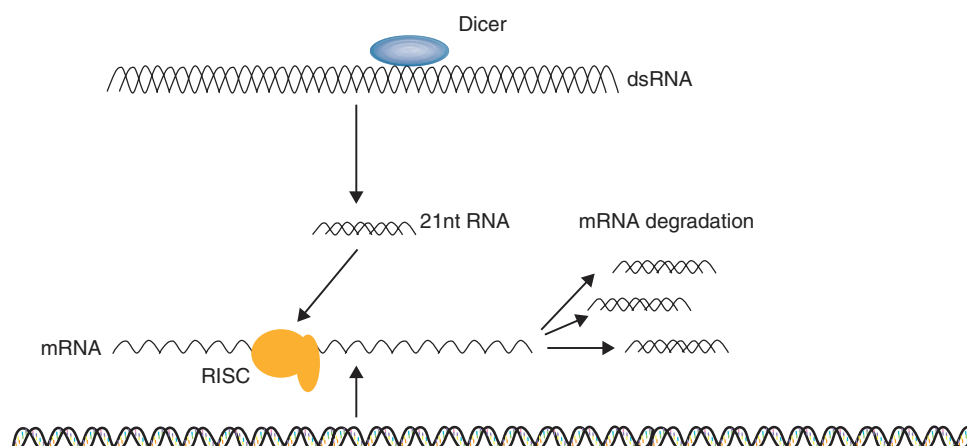
Figure 14-7

RNAi mechanism. In the initiation step, long dsRNA molecules are digested by the enzyme Dicer. Dicer activity leads to successive ATP-dependent cleavage events that degrade the RNA to 19-21 base pair (bp) duplexes (siRNA), each with 2-nucleotide 3′ overhangs. In the effector step, the siRNA molecules bind to a nuclease complex to form the RNA-induced silencing complex (RISC). Through base pair interactions, the RISC targets the homologous mRNA transcript and then cleaves the mRNA approximately 12 nucleotides from the 3′ terminus of the siRNA.

The exact mechanism of cleavage is unclear, but it is considered that RISC contains an RNase that is distinct from Dicer.[4,5]

In mammalian cells, the initiation step is bypassed because of the induction of the antiviral or interferon response pathways. However, as previously described, this can be overcome by direct introduction of siRNA molecules into the cell.

IMPACT OF RNA INTERFERENCE

The discovery that siRNA molecules can cause gene-specific silencing in a fashion that is almost 10 times more potent than conventional antisense technologies has immense implications for cancer research and therapeutics.[6] The use of transiently transfected siRNA in mammalian cells to rapidly create loss of function phenotypes is enhancing researchers' ability to dissect the molecular mechanisms of cancer.[7,8] This transient and specific way to inhibit gene function can be thought of in the same sense as genetic knockout mice, although a much simpler approach. Before the discovery of RNAi, gene function analyses in cancer cells were difficult. Gene disruption using homologous recombination is difficult in cancer cells because most cells have a low targeting frequency. Furthermore, the diploid nature of the cells means that two rounds of targeting must be conducted. Antisense and ribozyme techniques have been used, but these are considered unreliable and they certainly are less specific than RNAi. RNAi already

is proving useful for deciphering genetic pathways in cancer and providing insights into potential therapeutic targets.[9,10]

It took only 2.5 years from the description of RNAi in nematodes to publication of the first large-scale functional genomic screens in this organism. With the sequencing of the human and canine genomes, it would seem only a matter of time before similar studies are published for these species. These screens will provide fertile ground for discovery because many assays for gene function are available in tissue culture cells. Libraries of siRNAs may be used in these assay systems to identify novel pathways and genes involved in the cancer phenotype. Simple screens for cell proliferation inhibition or apoptosis may be used to identify cancer-associated genes. Also, this approach may be combined with proteomics to reduce the pool of candidate genes for screening. The potential obviously is enormous and perfectly complements the expanding knowledge of the mammalian genomes.

RNA INTERFERENCE AS THERAPEUTIC MODALITY IN CANCER

RNAi as a tool for specifically targeting gene expression lends itself well to a disease such as cancer, in which genes often are upregulated or inappropriately expressed, causing uncontrolled cell division. *In vitro*, several model systems have been used to demonstrate

the potential that RNAi may offer in a therapeutic setting.[11,12] Although the tyrosine kinase inhibitor STI 571 has shown enormous promise in the treatment of CML in humans, resistance to this drug has been demonstrated. The Bcr/Abl fusion gene, associated with the pathophysiology of CML, lends itself well as a target for RNAi. In a recent study, siRNA molecules specifically targeted to Bcr/Abl mRNA were shown to cause apoptosis in CML cells as effectively as STI 571.[13,14] This may provide an alternative treatment modality in patients resistant to STI 571. Furthermore, it has been demonstrated that STI 571 can cooperate with Bcr/Abl-targeted siRNA to produce synergistic killing of CML cells through apoptosis.[15] Interestingly, this study also demonstrated that siRNA-mediated cell death could be achieved in CML cells that had acquired resistance to STI 571.

The number of studies using siRNA to therapeutically target cancer cells is growing at an exponential rate. Proof of principle studies in human and murine cells have been published using siRNA to target major genetic players in cancer, such as E6/E7 (cervical cancer), bcl-2 (leukemia), K-RAS (pancreatic carcinoma), and cyclooxygenase-2 (COX-2) (ovarian cancer), to name a few.[16] The results are encouraging, but this process obviously requires a thorough understanding of the molecular pathologies in cancer, an understanding that is still lagging in veterinary oncology. However, obvious targets exist for siRNA in cancers of dogs and cats. Telomerase now is regarded as a universal marker of malignancy in dogs and humans (Section D describes how siRNA techniques are being used to target this enzyme).[17]

OBSTACLES TO CLINICAL APPLICATION

The ability to specifically target gene expression in cancer cells and cause sustainable transcriptional silencing has significant potential for new treatment approaches for cancer in the domestic species and humans. As with all new technology, many obstacles must be overcome before these novel biologics become accepted clinical practice. As with gene therapy, delivery is a major hurdle. Systemic oligonucleotide delivery has been achieved in rodent models, but the action of the oligonucleotides becomes localized to the liver.[18] Viral vectors have been the backbone of gene therapy techniques since the initial experimental gene therapy trials began 14 years ago. However, the clinical disadvantages of many of the earlier vectors precluded their widespread acceptance. In recent years, a resurgence of excitement has occurred regarding the clinical use of viral vectors, and some of these vectors are finding a place in RNAi delivery systems. In particular, the safety profiles of lentiviruses and adenoviruses have significantly improved. The engineering of both these types of
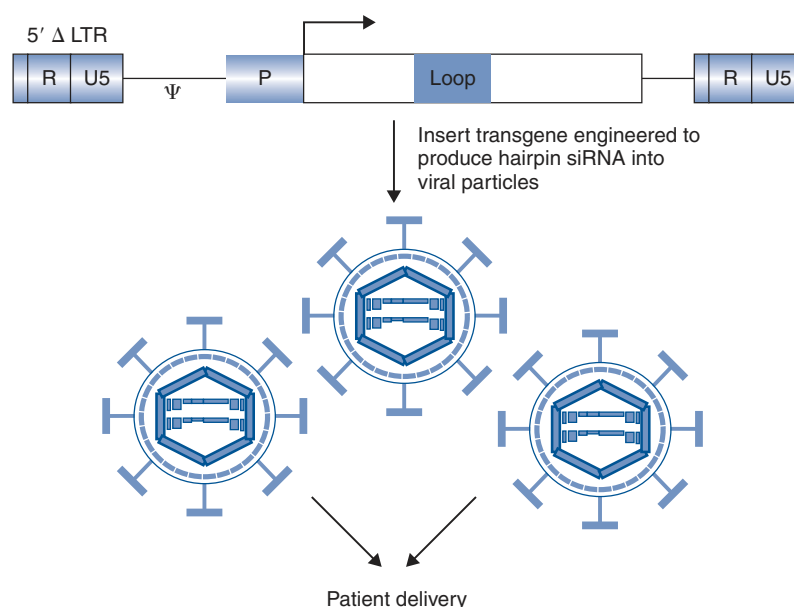
Figure 14-8

Lentiviral delivery of siRNA. Lentiviral vectors based on HIV-1 have been engineered to produce hairpin siRNA molecules under the control of a pol-III promoter (P). This vector system offers the potential for stable integration into target cells and continuous production of siRNA molecules.

vectors to produce hairpin siRNA molecules under the control of polymerase III (pol-III) promoters has allowed exploitation of the advantages of these vectors in siRNA-based protocols (Figure 14-8).[19-21] Although viral vectors have greater targeting efficiency than classical DNA methods, systemic delivery still is hampered by the immune and complement systems. Despite this, viral vectors are being developed that rely on cellular delivery to tumors systemically rather than injection of naked virus. As with gene therapy, parallel developments are occurring in the use of nonviral delivery systems for RNAi.[22] In particular, charge-reversible liposomes are being developed that can efficiently deliver nucleotides to areas of increased vascular permeability, such as tumors.

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SECTION D
Telomeres and Telomerase in Cancer Therapeutics

David J. Argyle

Despite advances in therapeutics, cancer remains a frequently fatal disease. A major challenge is the development of tumor-specific treatment strategies. Current methods, such as chemotherapy and radiotherapy, rely on a crude distinction between cancer cells and normal cells. However, with an increased understanding of the molecular events in the development of cancer, far more innovative and targeted approaches may be developed. From studies on humans and dogs, the enzyme telomerase has emerged as a central, unifying mechanism underlying the immortal phenotype of cancer cells and thus has become a candidate for differentiating between normal and cancer cells.[1] Telomerase is a ribonucleoprotein enzyme that maintains the protective structures at the ends of chromosomes, called *telomeres*. The telomerase holoenzyme is composed minimally of an RNA subunit (TR) and a catalytic protein subunit (telomerase reverse transcriptase [TERT]), and both components are essential for enzyme activity.[1-5]

TELOMERASE AND CANCER

It is becoming increasingly clear that many of the basic biologic processes in oncology are conserved among mammalian species, and as a result, the discipline of

comparative oncology is growing rapidly. The presence of telomerase activity in canine and feline tumor tissues has been reported by a number of investigators.[6-9] Taken together, these studies have analyzed more than 100 canine solid tumors for telomerase activity and have demonstrated that more than 95% of all canine cancers are associated with telomerase activity, whereas no telomerase activity could be detected in normal cells (apart from gametes and stem cells). The clear evidence linking telomerase activity to cellular immortalization and cancer development makes telomerase an attractive target for anticancer therapeutic research (Figure 14-9). Telomerase now is considered the most prevalent tumor marker in humans and dogs.

TELOMERASE AS A THERAPEUTIC TARGET

Telomerase has a clear role in cancer maintenance, providing the basic mechanism by which cancer cells attain immortality. The expression of telomerase in cancer cells maintains the chromosomal lengths and prevents the cell from entering senescence and cellular crisis. *In vitro* experimental evidence supports the hypothesis that inhibition of telomerase in cancer cells

leads to cell death independent of p53 status.[10] Numerous therapeutic strategies to inhibit telomerase have been suggested to translate these laboratory findings into clinical practice. Some of these strategies have included telomerase interactive compounds, such as peptide nucleic acids (PNAs), reverse transcriptase inhibitors, antisense molecules, dominant negative mutants of TERT, and ribozymes.[10-19] 39-Azido-29,3-dideoxythymidine (AZT) is a reverse transcriptase inhibitor that has been used in the treatment of acquired immunodeficiency syndrome (AIDS) and has been shown to inhibit telomerase activity in human cancer cells. However, it has proved difficult to reproduce in canine cells.[20]

In most studies, telomerase inhibition results in telomere instability and cell death. Although *in vitro* telomerase inhibition studies have shown promising results, such inhibition represents a challenge within the clinical setting. Inhibiting telomerase activity in cancer cells prevents maintenance of telomere lengths; however, tumor cells so treated still need to undergo sufficient cell divisions, with subsequent telomere shortening to a critical length, to trigger cell death. Because this predicted lag phase may be sufficient to allow tumor volume to increase, this approach is unlikely to benefit cancer patients with a large tumor burden.

Reverse transcriptase   RNA component

CAAUCCCAAUC

• Reverse transcriptase
  inhibitors: e.g. AZT
• RNAi

• Alkylation of guanine
• Antisense molecules
• RNAi

CAAUCCCAAUC   Telomerase reactivation

Targeted gene therapy

Immortal cancer phenotype
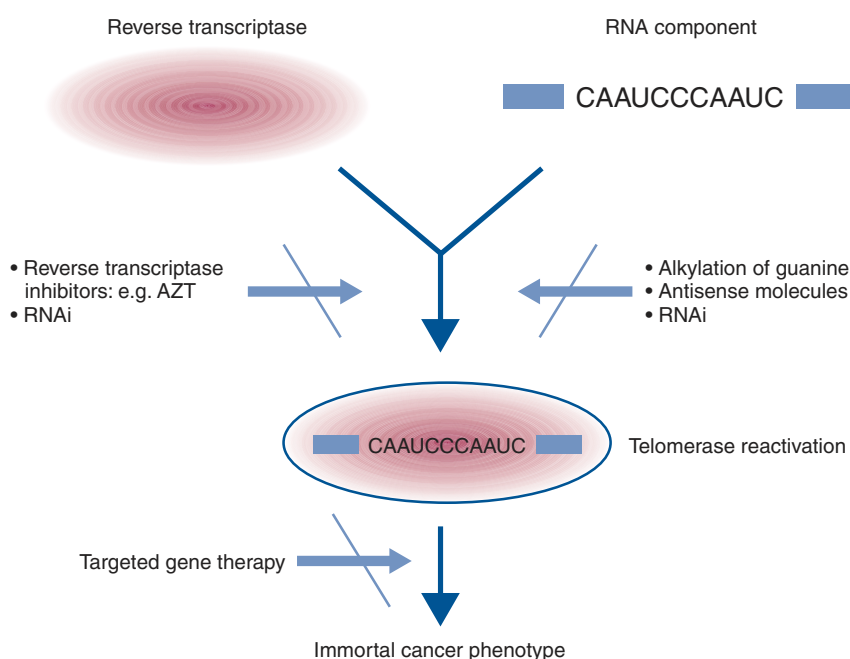
Figure 14-9

Possible targets of telomerase-based therapeutics. Telomerase is minimally composed of a reverse transcriptase component (TERT) and an RNA template. Both components are required for full telomerase activity and consequently represent targets for therapeutic intervention.

More recently, RNAi experiments have demonstrated the potential for this technology in telomerase inhibition in both humans and dogs.[21,22] Interestingly, these studies have shown that inhibition of telomerase actually causes rapid cell death despite the predicted lag phase. The reason has yet to be fully elucidated, but this offers promise in the development of RNAi for clinical benefit.

The greatest potential for telomerase inhibition likely will be in the adjuvant setting (i.e., in combination with current therapeutics such as chemotherapy, radiation therapy, and surgery) to specifically target residual disease or areas where micrometastatic disease is suspected. Furthermore, combining telomerase inhibition and tyrosine kinase inhibition has demonstrated synergy in killing tumors in rodent models.[23] Tumor cells with relatively short telomeres could be expected to respond faster to inhibition and cause cell death more quickly than tumor cells with longer telomeres, therefore exploitation of telomerase requires careful selection of patients who may benefit from the treatment. The chemotherapeutic agent cisplatin has been shown to potently inhibit telomerase activity and induce telomere loss in human testicular cancer cells.[24] Although cisplatin cannot be considered a selective telomerase inhibitor, it may prove useful in the primary treatment of tumors, followed by nontoxic telomerase inhibitors administered for a longer period, thereby overcoming the deleterious effects of long-term cisplatin administration. Cisplatin is a commonly used anticancer drug in canine oncology, and the investigation of the synergistic relationship between cisplatin and telomerase inhibitors in canine cancer cells needs to be established.

In addition to the potential for a lag phase in tumor cell death by telomerase inhibitors, a further concern lies in the potential for developing resistance. Most cancer cells attain immortality through the expression of telomerase. However, a small proportion of cancers do not express telomerase, but rather attain immortality through an alternative lengthening of telomeres (ALT) pathway.[25] This pathway is still not fully understood, but it is thought that telomeres are maintained through recombination events. Akin to the situation with conventional chemotherapeutic agents, it is possible that a cancer cell population that is continually exposed to a telomerase inhibitor may allow for selection of a population of cells with an ALT phenotype. However, evidence from rodent tumor models shows that metastasis relies on telomerase expression, and this may indicate that, even if a primary tumor becomes resistant to therapy, therapy may inhibit metastasis.[26]

TELOMERASE PROMOTER TARGETING

A different approach to the development of antitelomerase therapies may be offered by gene therapy. Instead of trying to inhibit telomerase per se, gene therapy capitalizes on the expression of telomerase to target expression of a novel transgene that can either kill the cancer cell or enhance the antitumor immune response to the cell. Strategies are developing to deliver therapeutic genes to cancer cells that are under the control of one of the telomerase promoter elements (Figure 14-10). Although the RNA subunit is ubiquitously expressed in most human normal somatic tissues, TERT expression is mainly confined to malignant tissues. This differential expression has led to a number of investigations of the ability of telomerase reverse transcriptase promoter elements to direct transgene expression specifically to tumor cells.[20] Indeed, these studies have demonstrated that the human telomerase promoter is sufficient to target cancer cells specifically. More recently, the canine promoter for TERT has been cloned and also has demonstrated potential as a targeting strategy for canine tumors.[26b]

TELOMERASE AS A UNIVERSAL ANTIGEN

Increasingly, data suggest that TERT can serve as a tumor antigen for a wide variety of cancer types.[27,28] Peptides derived from TERT are naturally processed by tumor cells, presented in association with major histocompatibility (MHC) class I, and serve as a target for antigen-specific cytolytic T cells (CTLs).[27] Telomerase activity is found in more than 85% of human cancers, and TERT expression correlates well with telomerase activity. A number of immunogenic peptides of TERT and peptide-specific CTLs have been described in humans. Induction of antimurine TERT immunity in mice vaccinated with dendritic cells transduced with murine TERT RNA has been achieved.[28] After vaccination, mice were partly protected from tumor challenge in three separate tumor models. Given the much broader expression of TERT in mice (compared with humans), a higher level of tolerance against TERT might be expected in the mouse; yet TERT immunity in these studies was easily achieved. Furthermore, the mice in this study did not suffer any autoimmunity against hemopoietic cells or other tissues that express murine TERT. Human TERT (hTERT)–specific CTLs have been generated *ex vivo* from cancer patients, which suggests that T cells specific to hTERT are neither fully deleted or irreversibly tolerized even in the setting of active neoplasia.[29-37]

At least three human clinical trials are underway in the United States to establish specific in vivo immune responses to TERT and to assess the toxicity of this approach. Initial results suggest that immunity to TERT
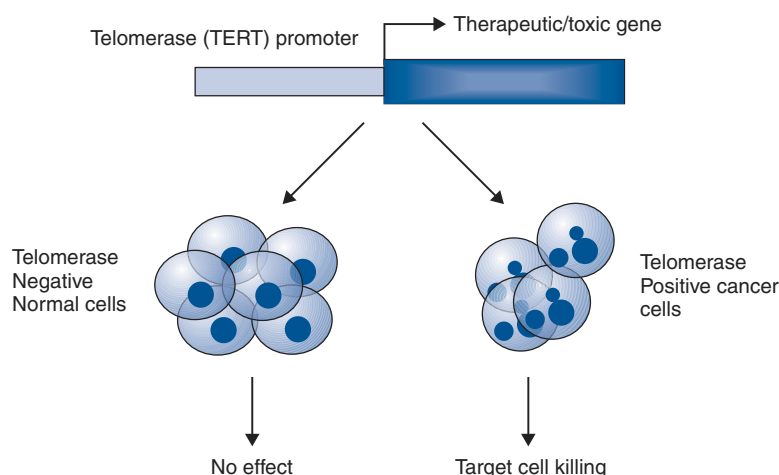
Figure 14-10

Targeted gene therapy using the telomerase promoter. The telomerase promoter is active only in cells that have telomerase activity. Consequently, the near-universal expression of telomerase in cancer cells, compared with normal cells, offers a method of transcriptionally targeted gene therapy.

can be generated safely and effectively. The overwhelming advantages of TERT as an antigen include the following:

- TERT can be broadly applied for a wide variety of tumors.
- Targeting TERT, which is critical for cancer survival, may help circumvent the problems of immune escape. Tumor downregulation of TERT as a means of immune escape might itself be incompatible with sustained tumor growth.
- T-cell responses to TERT may derive from a naïve repertoire and therefore are less likely to be limited by anergy or tolerance

Despite the antigenic nature of TERT, TERT vaccination strategies quite possibly will require linkage with other antigens or cytokine models or will be more effective in a xenogeneic setting (e.g., using murine TERT to generate immune responses in dogs).

TELOMERASE AS A DIAGNOSTIC TOOL IN CLINICAL ONCOLOGY

The near-universal expression of telomerase in cancer cells has made this enzyme the most prevalent tumor marker. Consequently, the detection of telomerase activity using the telomeric repeat amplification protocol (TRAP) assay may support a diagnosis of cancer. The TRAP technique can detect activity not only in solid biopsies and whole tumor specimens but also in tissue aspirates and malignant cytologic specimens. For example,

telomerase activity has been detected in urine samples from patients with bladder cancer, bronchial lavage samples from patients with lung cancer, and colonic washes from patients with colorectal cancer.[1,38-41] Although many studies have shown variable success in establishing a diagnostic role for telomerase activity, largely because of the infiltration of normal telomerase-positive cells (e.g., lymphocytes), telomerase levels may emerge as a useful diagnostic marker for particular cancer types. Telomerase activity has been detected in negative cervical smear samples associated with human papillomavirus infection. Similarly higher levels of telomerase activity have been detected in malignant pancreatic cancers compared with benign lesions.

Telomerase activity also may play a role in establishing a prognosis. Several studies have focused on the prognostic value of telomerase activity, which has been shown to be of value in a few cancer types, including neuroblastomas.[42] Clearly, careful, controlled studies in companion animals need to be performed to evaluate the potential prognostic and diagnostic role of telomerase in canine cancers.

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SECTION E
Targeting Angiogenesis and Tumor Vasculature

Ruthanne Chun and Douglas H. Thamm

Subsequent to seminal work by Folkman and others in the 1970s and 1980s, it has been unequivocally demonstrated that establishment of a vascular supply is obligatory for successful formation of a clinically relevant tumor.[1-3] Without the recruitment of its own vascular network, a tumor cannot exceed 2 mm in diameter, a size that would be clinically insignificant under most circumstances. A knowledge of the principles underlying the establishment of a vascular supply by tumors is critical to the understanding of tumor biology and has the potential to offer novel avenues for tumor control.

The vascular endothelium is a dynamic structure, and vascular endothelial cells have several important functions. They take up and then modify lipoproteins during transport in the artery wall. They maintain a permeability barrier by means of intercellular junctions, which widen when exposed to hemodynamic forces or vasoactive agents, such as histamine. Also in response to histamine, they release prothrombotic molecules, such as von Willebrand's factor (vWF), tissue factor, and plasminogen activator inhibitor, to prevent bleeding. They regulate egress of leukocytes by changing expression levels of adhesion molecules in response to inflammation, and they respond to hypoxia by producing growth factors such as VEGF and expressing receptor tyrosine kinases, allowing them to respond to paracrine angiogenic signals from the extracellular environment.

Angiogenesis and vasculogenesis are processes that result in the formation of vascular structures under normal and pathologic conditions. *Angiogenesis* is the ingrowth of blood vessels from previously existing vascular structures. *Vasculogenesis* is the *in situ* differentiation of progenitor cells into hemangioblasts, which subsequently differentiate into endothelial cells and form a primative capillary network. These progenitor cells, known as endothelial progenitor cells (EPCs), arise from the bone marrow and circulate in the peripheral bloodstream.[4] Vasculogenesis conventionally was thought to be limited to embryologic development, with angiogenesis occurring in both the developing embryo and postnatal life. However, recent reports describe EPCs in adult animals that are capable of differentiating into vascular endothelial cells, indicating that the capacity for vasculogenesis is not restricted to fetal development but is important in new vascular growth and repair throughout life.[4] Many factors regulate vasculogenesis and angiogenesis, and many factors have overlapping effects on the two processes. A third process, *vasculogenic mimicry*, has been described in certain tumor types.[5,6] In this process, blood-filled channels without endothelial linings are formed from tumor or stromal cells or both.

MOLECULAR AND CELLULAR ASPECTS OF ANGIOGENESIS

Angiogenesis is fundamental to reproduction, development, and tissue repair. However, the dysregulated growth of blood vessels plays an important role in such disease processes as diabetic retinopathy, rheumatoid arthritis, atherosclerosis, and psoriasis, as well as in malignant tumor growth and metastasis. Normally angiogenesis is tightly regulated by a balance of positive and negative growth factors. In neoplastic conditions, proangiogenic factors outbalance antiangiogenic factors, and pathologic angiogenesis ensues. Various growth factors and cytokines, such as VEGF, acidic and basic fibroblast growth factor (aFGF and bFGF), IL-8, transforming growth factors alpha and beta (TGF-α and TGF-β), PDGF, and prostaglandin E_2 (PGE$_2$), are known to induce angiogenesis.[7,8] Degradation of the extracellular matrix and basement membrane by matrix metalloproteinases (MMPs), leading to tissue remodeling and invasion, is crucial to both angiogenesis and metastasis.[9] Examples of antiangiogenic growth factors include IF-γ, thrombospondin-1, endostatin, and angiostatin.[10] These positive and negative regulatory factors can be released from tumor cells, endothelial cells, and macrophages in response to alterations in the immediate microenvironment (e.g., hypoxia, altered pH, or inflammation).

One of the central governors of the angiogenic balance in normal cells is the response to hypoxia, governed by the Hif-1 axis.[11] Oxygen is capable of diffusing a maximum of approximately 180 μm from a capillary before it is used completely; therefore tissues farther from a patent blood vessel are hypoxic. Hif-1, a heterodimeric transcription factor ubiquitously expressed in mammalian tissues, directly upregulates the expression of a large number of angiogenesis-related target genes, including those coding for VEGF, erythropoietin, and glycolytic enzymes. The abundance of Hif-1 protein in turn is regulated by the von Hippel-Lindau (VHL) tumor suppressor gene. Binding of Hif-1 to VHL is regulated by intracellular oxygen tension; high oxygen tension enhances VHL binding, which targets the VHL–Hif-1 complex for ubiquitination and proteolytic destruction. However, in hypoxia the interaction of VHL with Hif-1 is suppressed, allowing Hif-1 to persist.[12,13]

In addition to oxygen tension, a number of oncogenic signaling pathways can modulate Hif-1 stability under normoxic conditions. These include a variety of growth factors and cytokines that signal through the PI3K/AKT and MAP kinase pathways.[12,13] Furthermore, mutations

in the VHL gene that lead to altered Hif-1 binding have been shown to predispose to human tumors such as RCC and hemangioblastoma.[14,15]

Regulation of the balance of proangiogenic and antiangiogenic substances also is modulated by a variety of other mechanisms, including functional alterations in a variety of oncogenes and tumor suppressor genes. For example, inactivation of the tumor suppressor gene p53 has been shown to upregulate bFGF production and downregulate thrombospondin-1 production in tumor cells.[16,17]

The multiple steps required for successful angiogenesis include activation of endothelial cells, proteolytic degradation of the extracellular matrix and basal membranes, migration and proliferation of endothelial cells, endothelial tube formation, fusion, and reassembly of extracellular matrix. Based on this description, it is no surprise that studies have shown that increased angiogenesis not only enhances tumor growth but also promotes metastatic disease. (The mechanics of metastasis are discussed in Chapter 2.)

DIAGNOSTIC APPLICATIONS OF ANGIOGENESIS

In the study of angiogenesis, clinical applications have focused on the quantification of blood vessels or growth factors for prognostic purposes, the enhancement of angiogenesis (e.g., for revascularization after thrombotic disease), and the inhibition of angiogenesis. In oncology, active areas of research include the quantitation of microvessel density (MVD) and angiogenic growth factors and their receptors, as well as the inhibition of angiogenesis. In veterinary oncology, most publications have focused on MVD and characterization of angiogenic growth factors, although interest in antiangiogenic therapies is strong.

Microvessel Density

The hallmark MVD study was performed by Weidner and colleagues.[18] This group hypothesized that breast cancer lesions with little angiogenesis have a relatively low rate of metastasis, whereas lesions with more angiogenesis have an increased probability of metastasis. The investigators highlighted microvessels via immunohistochemical staining for factor VIII–related antigen and counted the numbers of vessels per 200× field. Both microvessel counts and vessel density grades were correlated with metastatic disease. The investigators found that for each 10-MVD increase in the count per 200× field, the risk of metastasis increased 1.59-fold. Many studies of human cancers have since corroborated these findings.[19-23] Studies of MVD in veterinary patients support the continued investigation of MVD as a prognostic tool (Table 14-3).

Measurement of Angiogenic Growth Factors

As mentioned previously, many growth factors promote angiogenesis. Multiple human studies that evaluated serum, plasma, and tissue growth factor concentrations have correlated these factors with the prognosis.[24-28]

TABLE 14-3   *Tumor Vascularization Findings*

Species/Tumor Type	Findings	Reference
Canine prostatic carcinoma	Mean microvessel density (MVD) is higher in malignancies than in benign prostatic epithelium.	57
Canine osteosarcoma	MVD is higher in dogs with gross pulmonary metastasis.	58
Canine mammary gland tumors	Mean MVD is higher in malignant than benign tumors. Tumors with a high MVD are more likely to recur. An inverse correlation exists between MVD and tumor differentiation.	59, 60
Canine cutaneous squamous cell carcinoma	An inverse correlation exists between MVD and tumor differentiation.	61
Feline mammary gland tumors	An inverse correlation exists between MVD and tumor differentiation and survival.	62
Canine transitional cell carcinoma	MVD did not decrease with response to therapy.	54, 63
Canine seminoma	MVD is higher in tumor compared with normal testicle.	64
Canine mast cell tumors	An inverse correlation exists between MVD and survival; a positive correlation exists between MVD and grade,	65

Because bFGF and VEGF play such an important role in angiogenesis and tumor progression, they both have been heavily investigated in human cancer patients. High concentrations of bFGF in blood and tumor tissue have been correlated with the prognosis in humans with cancer.[21,28] bFGF is cleared by glomerular filtration, therefore urine samples also may be assayed for this growth factor.[29] Compared with normal individuals, cancer patients have elevated VEGF concentrations in serum, plasma, and tumor tissues. VEGF exists as five different isoforms, some of which are soluble and some of which are bound to

extracellular matrix in tissues. Increased expression of VEGF in serum, plasma, and tumor tissues has been correlated with a poor prognosis in a variety of human malignancies.[21,26,28,30,31]

Table 14-4 presents a summary of the veterinary oncology studies of soluble and tissue levels of angiogenic growth factors.

Matrix Metalloproteases

The MMPs are a family of zinc-dependent extracellular matrix– and basement membrane–degrading enzymes

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TABLE 14-4   *Quantitative and Qualitative Studies of Soluble and Intratumoral Angiogenic Growth Factors in Veterinary Medicine*

Species/Tumor Type	Growth Factor and Assay	Findings	Reference
Canine transitional cell carcinoma	Urinary basic fibroblast growth factor (bFGF) via enzyme-linked immunosorbent assay (ELISA)	bFGF is higher in dogs with transitional cell carcinoma (TCC) than in normal dogs or dogs with cystitis.	66
Canine lung carcinoma, fibrosarcoma, osteosarcoma, pancreatic carcinoma, mast cell tumor	Vascular endothelial growth factor (VEGF) via reverse transcriptase polymerase chain reaction (RT-PCR) analysis, chicken chorioallantoic membrane assay	Canine VEGF is present in a variety of canine tumors and has the same biologic effect as human VEGF in standardized angiogenesis assays.	67
Canine basal cell tumors and cutaneous and digital squamous cell carcinomas	Tissue VEGF via immunohistochemical staining (IHC)	Expression is minimal in basal cell tumors; expression is higher in digital versus cutaneous squamous cell carcinoma.	68
Canine hemangiosarcoma	Plasma VEGF via ELISA	Tumor-bearing dogs had higher concentrations than normal dogs; concentrations did not correlate with stage of disease or tumor burden.	69
Multiple canine tumor types	VEGF via ELISA in effusions	VEGF is detectable in most effusions; no difference in concentrations is noted in neoplastic versus nonneoplastic effusions.	70
Feline mammary gland tumors	Tissue VEGF via IHC	Expression is higher in poorly differentiated tumors and is inversely correlated with survival.	62
Canine transitional cell carcinoma	Urinary bFGF via ELISA	Reduction after treatment is correlated with tumor response to piroxicam.	54, 63
Feline vaccine-associated sarcoma	bFGF, p53, and transforming growth factor-alpha (TGF-α) via IHC	bFGF is increased in younger cats with nodular tumor growth patterns; bFGF staining is correlated with TGF-α staining.	71
Canine seminoma	Tissue VEGF via IHC	Expression is higher in tumor versus normal testicle.	64
Various canine tumors undergoing radiation therapy	Plasma VEGF via ELISA	Tumor-bearing dogs had higher pretreatment VEGF levels than normal dogs. VEGF increased in dogs treated with low doses per fraction compared to dogs treated with a high dose per fraction.	72

that contribute to a wide range of pathologic processes. MMPs are produced as inactive proenzymes that are cleaved to active forms. Activation occurs through a variety of different mechanisms, growth factors, and other proteases. Concurrent production of endogenous tissue inhibitors of MMPs (TIMPs) also helps control the amount of MMP-induced matrix degradation. The ratio of active enzyme to TIMPs allows for the appropriate amount of degradation of extracellular matrix components. These enzymes are essential for successful tumor angiogenesis, invasion, and metastasis. A wide variety of MMPs are under investigation, but in veterinary oncology the gelatinases (MMP 2 and MMP 9), which cleave type IV collagen (the main component of basement membrane), have been the main focus of investigation.

Lana and colleagues compared the activity of MMPs in 30 canine OSA samples to that in unaffected stromal tissue (muscle).[32] Bands correlating with human MMP 2 and MMP 9 were detectable in canine neoplastic tissue, and significantly more gelatinolytic activity was noted in tumor tissue than in stromal tissue, which suggests that MMPs are involved in tumor growth and metastasis. Leibman and coworkers[33] reported on the activity of MMP 2 and MMP 9 in canine cutaneous MCTs. Proenzyme MMP 9 activity was significantly higher in grade III tumors than in grade II tumors, and a dramatic difference was seen between tumor tissue and normal stroma with regard to both active MMP 2 and MMP 9, which suggests that MMPs play a role in the growth and progression of canine MCTs.

Jankowski and others investigated MMP activity in tumor, stromal tissue, and serum from 49 cats with various malignancies. Serum also was collected from 44 healthy cats. Measurable concentrations of MMP 2 and MMP 9 were found in all tissues sampled. The mean MMP concentrations for sarcomas and carcinomas were higher than in paired stromal tissue, although not all differences were significant. Serum MMP concentrations were higher in tumor-bearing cats compared with normal cats. The prognostic significance of MMP expression in feline malignancies is unknown.

Measurement of Angiogenic Inhibitors

Angiogenic inhibitors such as angiostatin and endostatin have not been well studied in veterinary oncology. Antiangiogenic factors are of interest in oncology patients because of the clinical observation in some cases of rapid growth of previously dormant metastases after removal of the primary tumor. Primary tumors may release antiangiogenic substances, such as endostatin, which may be capable of controlling distant metastases.[35,36]

Angiostatin is an internal fragment of plasminogen, the precursor of plasmin, an enzyme that dissolves fibrin clots. Proteinases from macrophages, as well as MMPs and plasmin, cleave angiostatin from plasminogen. Pirie-Shepherd and colleagues[37] investigated angiostatin in dogs with OSA and prostate cancer.[37] They pooled the urine of four dogs with OSA before and 1 to 12 weeks after amputation and assessed angiostatin presence by Western analysis. The urine samples obtained before amputation contained angiostatin, whereas samples collected after amputation did not. Canine prostate tumor cells also were demonstrated to convert plasminogen to angiostatin *in vitro*.

The antineoplastic properties of endostatin, a proteolytic fragment of collagen XVIII, include suppression of endothelial gene expression, inhibition of endothelial migration, and induction of apoptosis in cancer and endothelial cells. Endostatin is produced by a variety of cells, including platelets, endothelial cells, and tumor cells. Rossmeisl and coworkers[38] used a commercially available enzymatic immunoassay to measure endostatin in canine serum. A total of 53 healthy dogs and 38 tumor-bearing dogs (17 with lymphoma [LSA] and 21 with HSA) were evaluated. Mean serum endostatin concentrations were significantly higher in dogs with neoplasia than in healthy dogs. However, endostatin levels also were higher in older dogs, and the mean age of the neoplasia group was 8.6 years, compared with 5.5 years for the control group.

ANTIANGIOGENIC THERAPY

The endothelium serves as an attractive target for cancer therapy. Functional impairment of a single small capillary has the potential to affect the function of hundreds or thousands of tumor cells adversely. Furthermore, endothelial cells are thought to be "genetically stable" compared to tumor cells and therefore may be less able to develop resistance to therapy as a result of rapid mutation and selection for resistance.[39] A number of investigators have demonstrated phenotypic differences between normal ("resting") endothelium and active ("angiogenic") endothelium associated with tumor vasculature.[40-45] These differences include altered surface expression of adhesion molecules and growth factor receptors, enhanced survival, and upregulation of motility- and invasion-associated genes. These phenotypic changes may translate into differential sensitivity to antiangiogenic strategies, or they may present novel targets for tumor-directed therapy.

Interestingly, many studies have suggested that "standard" cytotoxic therapies, such as traditional antineoplastic chemotherapy and radiation therapy, may exert some of their effects through targeting of the endothelial compartment.[10,46-50] A continuous, low-dose (i.e., metronomic) dosing schedule of chemotherapy appears to increase the efficacy of targeting tumor vasculature rather than the malignant tumor cells.[51] Benefits of

metronomic chemotherapy include decreased patient toxicity and decreased to no induction of target cell (e.g., vascular endothelial cell) resistance because of inherent genetic stability.[51] Metronomic dosing strategies (e.g., small, daily doses of chemotherapy that result in inhibition of the vascular endothelial cells rather than tumor cells), which have been shown to be efficacious in mouse models of neoplasia,[49] have not yet been reported for veterinary patients and have yet to be evaluated thoroughly in clinical trials in humans.

The tumor vasculature also can be targeted for therapy by a variety of other strategies (Table 14-5).

Antiangiogenic therapies have not been well studied in veterinary medicine. A large, randomized, multiinstitutional study evaluating standard chemotherapy with or without an MMP inhibitor in canine LSA and OSA has not been published. However, no significant improvement in survival was identified between dogs treated with conventional therapy alone and those treated with conventional therapy plus the MMP inhibitor (Personal communication; Dr. Greg Ogilvie).

Minocycline has been touted as an antiangiogenic agent having potent anticollagenase activity.[52] Sorenmo and colleagues[53] prospectively treated 18 dogs with hemangiosarcoma of various stages and locations with surgery, doxorubicin, cyclophosphamide, and minocycline.[53] No statistical difference in survival was found between the dogs treated in this single-arm study and historical controls treated with surgery and chemotherapy alone.

The COX-2–inhibiting nonsteroidal antiinflammatory drug piroxicam is regarded as a potential antiangiogenic agent, because PGE_2 has proangiogenic effects. Mohammed and coworkers studied the effects of piroxicam on tumor response, apoptosis, and angiogenesis in canine transitional cell carcinoma (TCC).[54] The mechanisms by which COX inhibitors exert antitumor effects are not completely defined but are believed to involve immunomodulation, antiangiogenesis, and/or induction of apoptosis. In this study, the effect of piroxicam on tumor response, apoptotic index, proliferative index, COX-2 expression, PGE_2 concentration, tumor MVD, and urine bFGF and VEGF concentrations was studied in pet dogs with naturally occurring, invasive TCC of the urinary bladder. Piroxicam caused reduction in tumor volume in 12 of 18 dogs (although only six had a partial remission or better), and this was strongly associated with induction of apoptosis and a reduction in urine bFGF concentration. Unfortunately, the criteria used to define tumor response were not stringent, and the results remain questionable.

Although much remains to be learned about angiogenesis in veterinary oncology patients, it is clear that blood vessel formation is a major factor in the success of tumor growth and metastasis. Antiangiogenic therapies such as inhibition of growth factor receptors and targeting of growth factors no doubt will be available in the future, but they likely will be most efficacious when used in conjunction with more conventional treatments.[55,56]

TABLE 14-5   Potential Strategies for Clinical Antiangiogenic Therapy

Strategy	Phase of Development	Reference
Antiangiogenic factor/receptor blockade		
Small molecule kinase inhibitors	Middle and late stage clinical trials	73-75
Antibodies	Drug approved	55, 76, 77
Soluble decoy receptors	Preclinical evaluation	78, 79
Exogenous administration of angiogenesis inhibitors		
Endostatin, angiostatin	Early and middle stage clinical trials	80, 81
Thrombospondin-1	Preclinical evaluation	82, 83
Matrix metalloproteinase (MMP) inhibitors	Early and middle stage clinical trials	84, 85
Cyclooxygenase-2 (COX-2) inhibitors	Numerous drugs approved	54, 63, 86, 87
Drugs targeting angiogenic vasculature	Preclinical evaluation or early clinical trials	88-91
Vaccines targeting angiogenic endothelium	Preclinical evaluation or early clinical trials	92-96
Vascular toxins	Early and middle stage clinical trials	97, 98
Metronomic chemotherapy	Drugs approved (efficacy unknown)	99-101
Hif-1 inhibitors	Preclinical evaluation or early clinical trials	102, 103
Genetically modified endothelial cells	Preclinical evaluation	104

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SECTION F
Novel and Emerging Therapeutic Targets

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The recent explosion in available tumor bioinformatics, rational and combinatorial drug design, and high-throughput drug screening has resulted in a massive increase in potential therapeutic targets and anticancer treatment strategies. An exhaustive survey of all potential novel targets for cancer therapy would be impossible; therefore, this review presents only a brief overview of some of the more promising and well-developed "druggable" targets that have been discovered and, where applicable, their potential use in veterinary oncology.

DNA METHYLATION

In addition to the information encoded within the genome sequence, it has been shown that epigenetic changes are of great importance in the modification and maintenance of gene expression. These changes take place through a number of mechanisms, including polymerase enzyme modulation, chromatin condensation, and DNA methylation. Mammalian DNA is methylated at cytosines within cytosine-guanine (CpG)

dinucleotide sequences. During tissue differentiation, the methylation pattern is one governor of tissue-specific gene expression and therefore, ultimately, of phenotype.[1-3]

Two different methylation-related phenomena have been identified in cancer. Tumor cell DNA has been shown in both dogs and other mammals to be globally hypomethylated,[4,5] specifically in pericentromeric satellite sequences. This may lead to decreased genome stability and an increase in the incidence of oncogenic chromosome defects. Indeed, the purposeful induction of genomic hypomethylation by reduction in germline DNA methyltransferase-1 (DNMT1) levels in genetically engineered mice is associated with a high incidence of T-cell lymphomas displaying trisomy 15.[6] Cancer cells also acquire sequence-specific promoter hypermethylation and transcriptional repression in normally unmethylated regions, several of which have been shown to be associated with known tumor suppressor genes, including Rb, p16, p73, and the VHL protein,[1,2,7-9] or other important tumor-associated genes, such as E-cadherin and estrogen and retinoic acid receptors.[10]

The methylation of DNA is controlled by four known DNMTs, of which DNMT1 may be the most important in cancer.[1-3] A variety of agents have been shown to inhibit DNMT function. The two best studied are 5-azacytidine and 5-aza-deoxycitidine (decitabine), nucleoside analogs that incorporate into DNA and inhibit DNMT activity but allow replication to proceed. A large number of single agent clinical trials with these drugs have been reported, and significant activity has been demonstrated in hematopoietic neoplasia, leading to approval by the U.S. Food and Drug Administration (FDA) for the use of 5-azacytidine in the treatment of myelodysplastic syndrome.[11-13] Encouraging response rates to decitabine also have been seen in patients with imatinib-refractory CML.[14] Results in advanced solid tumors have been generally disappointing,[15,16] although studies of decitabine use in combination with standard antineoplastic drugs are underway.[17] One drawback of these drugs is the requirement for DNA replication for activity. Various other drugs have DNMT inhibitory activity in nonreplicating cells, including green tea polyphenols[1] and a marine product of the psammaplin class.[18] The latter, a synthetic derivative that concurrently inhibits histone deacetylase, is in human clinical trials.[19] Interestingly, the commonly used cardiac medications procainamide and hydralazine also have demethylating activity,[10,20] and a clinical trial recently demonstrated alterations in promoter methylation and reactivation of silenced genes after administration of well-tolerated doses of hydralazine to human patients with cervical cancer.[21] Procainamide and hydralazine have a long track record of use in veterinary medicine and as such could serve as interesting and readily available drugs for the initial evaluation of methylation inhibition in veterinary cancer patients.

Two potential problems exist regarding widespread clinical implementation of DNMT inhibitors for cancer treatment. As discussed previously, induction of long-term, genome-wide hypomethylation could reduce chromosome stability, leading to potentially tumorigenic chromosome rearrangements.[3] A recently described new class of DNMT inhibitor is capable of demethylating and reactivating tumor suppressor genes without altering pericentromeric methylation status, which could minimize this risk.[22] Demethylation also could trigger the reactivation of genes, promoting a more aggressive or metastatic phenotype.[3] In support of this theory, treatment of nonmetastatic breast cancer cells with 5-azacytidine was shown to upregulate expression of urokinase-like plasminogen activator, an enzyme important in tumor invasion and metastasis, leading to enhanced metastatic potential.[23]

HISTONE DEACETYLASE

Another critical determinant of gene expression is the condensation of chromatin in the form of heterochromatin, which results in transcriptional silencing and enhanced genome stability. This is accomplished by a number of pathways, one of which is the acetylation and deacetylation of histones, controlled by histone acetyltransferases and histone deacetylases (HDACs).[24] The HDACs specifically maintain chromatin in a condensed form and can associate with specific transcription factors, resulting in transcription repression. The acetylation of histones may be the key to regulating the expression of genes associated with cellular proliferation, differentiation, and survival both in development and carcinogenesis.[25,26] Initial studies suggested that histone acetylation reduced electrostatic charge interactions between histones, leading to chromatin decondensation, but more recent work has suggested that HDAC's effect may be indirect, through modulation of other proteins important to chromatin condensation, including DNMT1.[27] Induction of HDAC expression, leading to transcriptional repression, is a common feature in human cancers such as colon cancer,[28] and it negatively regulates the expression of several tumor suppressor genes, including p53 and VHL.[29] The pattern of global histone modification recently was shown to correlate with outcome in humans with low-grade prostate cancer.[30]

Pharmacologic inhibition of HDAC can affect multiple facets of the malignant phenotype. HDAC inhibition reduces colon carcinogenesis in the APC mouse model.[28] Angiogenesis can be repressed through upregulation of VHL and subsequent inhibition of

hypoxia-inducible factor-1-alpha (Hif-1α) function and VEGF production,[29,31,32] decreased expression of other proangiogenic factors (e.g., bFGF, angiopoietin-2, and Tie-2),[31,32] inhibition of endothelial nitric oxide synthase and endothelial cell proliferation and tube formation,[33,34] and inhibition of the commitment of endothelial progenitor cells to the endothelial lineage.[35] Inhibition of HDAC can enhance apoptosis in tumor and endothelial cells[32,36-38] and directly inhibit tumor cell proliferation.[37,39-41] Consistent with its role as a transcription repressor, inhibition of HDAC has been shown to induce differentiation in thyroid and prostate cancer, neuroblastoma, and the leukemias.[42-46] HDAC inhibition dramatically enhances the *in vitro* and *in vivo* efficacy of multiple standard cytotoxic therapies and of novel antibodies and small molecules.[27,32,38,47-52] Recently, hepatoma cells, but not normal hepatocytes, have been shown to upregulate the expression of natural killer (NK) cell receptor NKG2D ligands on their surfaces after exposure to HDAC inhibitors, rendering them more sensitive to NK cell–mediated destruction.[53]

A handful of human phase I studies[54-59] and phase II studies[60-62] in NSCLC, leukemias, and myelodysplastic syndromes, as well as two studies in combination with chemotherapy[63,64] evaluating five different HDAC inhibitors, have been published. Antitumor activity has been observed in myelodysplastic syndrome/AML, T-cell lymphoma, and lung cancer,[60-62,65] and proof of target has been shown in multiple studies by demonstrating increases in acetylated histones in peripheral blood lymphocytes or tumor tissue.[54,57,63,65] Interestingly, one study showed a correlation between response to combination carboplatin/paclitaxel/HDAC inhibition and a greater than 1.5-fold increase in peripheral blood acetylated histone H3.[63]

A single case report describes administration of the HDAC inhibitor suberoylanilide hydroxamic acid to a dog with microscopic hemangiosarcoma.[66] Although the drug apparently was well tolerated, the dosage was empirically chosen, there was no pharmacokinetic analysis or demonstration of target inhibition, and the drug's impact on the course of disease could not be unequivocally determined.

The commercially available anticonvulsant drug valproic acid (VPA) has been shown by many investigators to function as an HDAC inhibitor; it is capable of inhibiting tumor cell invasion, proliferation, and angiogenesis and of enhancing chemosensitivity.[24,27,43] Information on the use of VPA as an antiepileptic drug in dogs and canine pharmacokinetic and toxicologic data have been generated after oral administration.[67-69] VPA therefore is a readily available drug that could be used to begin the evaluation of HDAC inhibition as a treatment for canine cancer.

PROTEASOME

The abundance of cellular proteins is tightly controlled at the levels of both production and destruction. Protein production can be modified at the transcriptional and posttranscriptional level, and therapies based on these approaches are relatively abundant. Until recently, relatively little attention has been paid to the manipulation of protein degradation as a therapeutic modality. The ubiquitin-proteasome pathway (UPP) is responsible for the degradation of most intracellular proteins and is responsible for the regulation of many proteins that play key roles in cancer.

The 26S proteasome is a large, multiprotein complex with ubiquitin recognition domains, which bind ubiquitinated proteins tagged for degradation, and proteolytic domains with trypsinlike, chymotrypsin-like, and caspaselike activity, which degrade proteins into short polypeptide sequences.[70] 26S is responsible for the degradation of a variety of proteins that effect cell cycle regulation, angiogenesis, apoptosis, and sensitivity to chemotherapy and radiation therapy (Table 14-6).[70-72]

Tumor cells generally are more sensitive to the effects of proteasome inhibition than are normal cells. Various studies have demonstrated a threefold to fortyfold increase in susceptibility to proteasome inhibitor–associated apoptosis when comparing tumor cells to corresponding normal tissues.[72-76] The mechanisms for this differential sensitivity are unclear, but proliferating cells generally appear more sensitive than quiescent cells.[70,72] In addition, dysregulation of UPP function appears to occur in many cancer cells, thus potentially rendering them more sensitive to inhibition.[72,77] Recent studies implicate profound upregulation of the proapoptotic factor Noxa as a key mediator of proteasome inhibitor–induced tumor cell apoptosis.[78,79]

Although a number of chemicals appear capable of proteasome inhibition *in vitro*, only the boronic acid derivatives appear suitable for clinical use and only one drug, bortezomib (Velcade; PS-341), has entered human clinical trials to date. Six phase I and eight phase II trials in soft tissue sarcoma, prostate carcinoma, lymphoma, multiple myeloma, neuroendocrine tumors, RCC, and melanoma have been reported to date. Despite the drug's very short plasma half-life, pharmacodynamic analysis has demonstrated inhibition of proteasome activity in peripheral blood mononuclear cells for up to 72 hours after a single dose.[72] Meaningful antitumor activity has been seen in patients with multiple myeloma[80-83] and other hematopoietic neoplasia,[84-86] and the impressive responses seen in patients with multiple myeloma have led to accelerated FDA approval of bortezomib for use in the treatment of this disease. Less activity has been seen in solid tumors to date.[87-90] Combination studies with melphalan,[91] doxorubicin/dexamethasone,[92] and

TABLE 14-6   Molecular Targets and Consequences of Proteasome Inhibition

Process	Proteins Degraded by Proteasome	Cellular Consequences
NF-κB activation	IκB	Accumulation of IκB inhibits nuclear translocation and activity of NF-κB, leading to decreased proliferation, survival, invasion, and angiogenesis.
Apoptosis	p53, Bax, tBid, Smac, JNK, Noxa	Accumulation of these proteins directly or indirectly promotes apoptosis through various pathways.
Cell cycle regulation	p21, p27, other CDK inhibitors, cyclins, p53	Accumulation of CDK inhibitors can cause cell cycle arrest and apoptosis. The dysregulated elevation of multiple cyclins can send contradictory signals to the cell, resulting in apoptosis.
Signal transduction	MKP-1 phosphatase	Accumulation dephosphorylates p44/42 MAP kinase, leading to decreased MAPK pathway signaling, proliferation, and survival, with or without angiogenesis.
Oncogenic transformation	c-Fos, c-jun, c-myc, N-myc	The mechanism by which an overabundance of these proteins exerts an antitumor effect is unclear.
Unfolded protein response	Various damaged or misfolded proteins	Accumulation of damaged proteins leads to endoplasmic reticulum stress and apoptosis.
Chemotherapy/radiation sensitivity	IκB, P-glycoprotein (P-GP), topoisomerase-IIα (TOP- IIα), DNA damage repair enzyme downregulation	NF-κB is induced in response to DNA damage; normal proteasome function is required for correct folding of P-GP; downregulation of TOP-IIα may reduce sensitivity to doxorubicin.

From Adams J: The development of proteasome inhibitors as anticancer drugs, Cancer Cell 5:417-421, 2004;
Rajkumar SV, Richardson PG, Hideshima T et al: Proteasome inhibition as a novel therapeutic target in human cancer, J Clin Oncol 23:630-639,
2005; and Voorhees PM, Dees EC, O'Neil B et al: The proteasome as a target for cancer therapy, Clin Cancer Res 9:6316-6325, 2003.

pegylated liposomal doxorubicin[93] have been reported, and many others are underway. Although toxicology studies with bortezomib have been performed in dogs,[94] no information is available to date regarding the safety or biologic effect of proteasome inhibitors in veterinary patients or tumor cells.

HEAT SHOCK PROTEIN 90

Given the complex nature of cancer and the multiple pathways that can be subjugated to contribute to the malignant phenotype, an optimal cancer drug might target a variety of oncogenic pathways simultaneously. One molecular target that has the potential to interrupt a wide variety of pathways important in cancer is heat shock protein 90 (HSP90), a molecular chaperone responsible for the conformational maturation of many proteins involved in diverse oncogenic activities such as cell adhesion, migration, and invasion; signal transduction; cell cycle progression; angiogenesis; and survival (Table 14-7). HSP90 and other chaperones are responsible for ensuring the correct folding of their client proteins, as well as for preventing aggregation of these proteins.[95] Misfolding and aggregation of proteins lead to ubiquitination and proteasomal destruction, resulting in proteins with diminished function and greatly shortened half-lives.[96] Although several classes of compounds are capable of inhibiting HSP90 chaperone function,[97-99] the best studied are the ansamycin antibiotics of the geldanamycin class.

Many HSP90 inhibitors appear to demonstrate significant preferential activity against malignant versus normal somatic cells. The HSP90 derived from most tumor cells has a binding affinity for 17-allylaminogeldanamycin (17-AAG) approximately 100-fold higher than HSP90 derived from normal cells. This may occur as a result of overaccumulation of mutated, misfolded, and overexpressed signaling proteins in tumor cells, leading to increased HSP90 chaperone activity and a greater proportion of the molecule in the bound, active, and 17-AAG–sensitive state.[100]

Tumor cells display considerable variation in sensitivity to HSP90 inhibition. Although the mechanisms

TABLE 14-7 Molecules Targeted by Inhibition of Heat Shock Protein 90		
Process	**Targets**	**Reference**
Invasion and migration	Urokinase-like plasminogen activator,* FAK phosphorylation	129-131
Cell cycle progression	Cyclin D3, CDK4	132
Signal transduction	AKT, Kit, Raf-1, EGFR, HER2, Jun, Lyn, Src, IGF-1R, PDGFR, Met, Bcr/Abl, ILK, androgen receptor, progesterone receptor, glucocorticoid receptor	101, 102, 104, 119-122, 124, 125, 132-136
Hypoxic response/angiogenesis	Hif-1, VEGF, Glut-1, nitric oxide synthase	97, 98, 101, 113, 114, 120, 137
Antiapoptosis	Wild-type and mutant p53, survivin	99, 115-118, 134, 138
Cell senescence	Telomerase	139, 140

Urokinase-like plasminogen activator activity appears to be inhibited by geldanamycin class drugs through a mechanism other than inhibition of heat shock protein 90.

CDK, Cyclin-dependent kinase; EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor ligand; PDGFR, platelet-derived growth factor receptor; HiF-1, hypoxia-inducible factor 1; VEGF, vascular endothelial growth factor.

underlying this differential sensitivity are incompletely described, some important characteristics include reliance on certain kinase cascades, expression of apoptotic and cell cycle regulators, and P-GP expression.[101]

Many receptor tyrosine kinases targeted by the geldanamycins may have an important role in canine and feline tumors. For example, they are capable of inhibiting the function of mutant and wild-type c-Kit,[102] important in canine mast cell neoplasia;[103] c-Met,[104] expressed in multiple canine tumor types;[105,106] PDGFR,[107] expressed in feline vaccine-associated sarcoma and canine osteosarcoma[108,109]; and IGF-1R,[102] which is expressed and functional in canine osteosarcoma and melanoma.[110-112] The geldanamycins are likewise able to attenuate the function of the Hif-1 protein, a key transcription factor responsible for sensing and responding to hypoxia and activating the angiogenic switch.[97,113,114] The geldanamycins also are able to deplete key antiapoptotic proteins such as mutant p53 and survivin,[115-118] contributing to enhanced *in vitro* sensitivity to standard cytotoxic therapies such as radiation therapy and chemotherapy when used in combination.[119-124]

HSP90 inhibitors such as 17-AAG target a virtual who's who of oncogenic signaling targets and therefore are capable of inhibiting a plethora of important pathways in tumor biology. However, under certain circumstances, drugs such as 17-AAG may have negative effects. For example, 17-AAG has been shown to protect colon carcinoma cells from cisplatin-mediated toxicity,[125] whereas it has additive or synergistic activity when combined with cisplatin against human neuroblastoma and osteosarcoma cells.[119] In addition, although 17-AAG inhibited primary tumor formation, it potentiated bone-specific mammary carcinoma metastasis by enhancing osteoclastogenesis in one murine model.[126]

The impressive preclinical data generated with compounds such as 17-AAG have led to published phase I human clinical trials that evaluated daily and once weekly administration schemes.[127,128] Dose-limiting toxicities have been primarily hepatic and gastrointestinal, and the peak plasma concentrations achieved are in the range associated with *in vitro* biologic activity. Evidence of biologic effect, in the form of upregulation of HSP70 chaperone expression in peripheral blood cells, was seen in both studies. Alternate administration schemes and studies in combination with chemotherapy are underway. Clinical or laboratory evaluation of HSP90 inhibitors in veterinary tumors has yet to be reported.

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Miscellaneous Treatments for Solid Tumors

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SECTION A
Cryosurgery

Stephen J. Withrow

INTRODUCTION

Cryosurgery is the controlled use of cold temperature to induce cellular death. After an initial intense enthusiasm for its use on a variety of neoplastic and non-neoplastic conditions, its use has settled down to more selected conditions in selected sites.[1] Cryosurgery can be a useful addition to conventional therapy due to its speed, predictability, and avoidance of general anesthesia in many settings.

SOURCE OF CRYOGEN AND EQUIPMENT

Many cryogens exist to induce cold temperature in tissue. The most commonly used are nitrous oxide (N_2O) and liquid nitrogen (N_2). The advantages and disadvantages of these cryogens are summarized in Table 15-1.

Liquid nitrogen is more difficult to work with, since it evaporates slowly and usually requires transfer from the Dewar (storage container) to the applicator. Nitrous oxide utilizes the same blue tank employed for anesthesia purposes and does not evaporate between uses. Cost per procedure for each cryogen is comparable.

Nitrous oxide is suitable for small (<1 cm) lesions but is somewhat restrictive due to its higher temperature, slow speed of freezing, depth of penetration, and lack of effective spray capabilities. On the other hand, it is acceptable for the more common small benign lesion that is frequently treated (skin and eyelid). If one intends to treat larger than 1-cm diameter lesions

having a rich blood supply or that are invading bone, N_2 is a superior cryogen in spite of its more difficult handling properties.

Nitrous oxide machines are usually portable on wheels with the tank attached and have a variety of probe sizes and configurations. A spray orifice is provided in some models, but this is very ineffective compared to N_2. Certain machines also have a thermistor incorporated into the probe head that documents the probe temperature (not necessarily tumor temperature). Rewarming of the probe tip is also available on some models to allow more rapid detachment of the probe from the tumor after freezing.

Liquid nitrogen machines may be small and portable in a briefcase or can be purchased with a combination of a Dewar and applicator. N_2 may be applied with various probes or with a fine mist or spray. Spray freezing is capable of faster and deeper penetration of tissue per time of application than is probe freezing. Additionally, it has advantages in treating lesions that are not spherical.

It is advisable to purchase a temperature monitoring device with thermocouple needles to monitor temperature in the tumor and normal tissues to be protected. This monitoring is especially important as one is learning the technique and is always used in monitoring temperature of critical normal tissue (nerves, rectal mucosa, joint capsule, etc.).

MECHANISM OF CELL DEATH

Much literature exists on the mechanisms of cell death after freezing.[2] In summary, two basic events lead to death of a cell after freezing:
1. *Direct cellular death*. This occurs in the first few minutes after freezing and results from ice crystal disruption of cellular membranes, electrolyte changes, alteration of cellular proteins, and thermal shock.

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TABLE 15-1   Comparison of N$_2$ and N$_2$O

	Liquid Nitrogen (N$_2$)	Nitrous Oxide (N$_2$O)
Lowest temperature	−196° C	−89° C
Availability	Welding firms, medical supply firms, artificial insemination stations	Medical supply firms
Storage	Requires a Dewar (storage container) and evaporates at 1% per week (as for anesthesia purposes)	Contained in standard blue tank
Probe freezing	Yes	Yes
Spray freezing	Yes	Poor
Depth of freezing	Deep	Shallow

2. *Vascular collapse*. Small blood vessels and particularly capillaries are irreversibly damaged after freezing and subsequently collapse, inducing hypoxia and infarction of frozen tissue. Large and medium-size arteries and, to a lesser extent, large veins are resistant to permanent injury.

The most important factors that influence cell death or survival after freezing are the speed of freezing and thawing, the number of freezes applied, and the coldest temperature reached throughout the tumor.

Fast freezing and slow thawing are most lethal to mammalian cells. The time to reach the coldest temperature is influenced by many factors, including cryogen (liquid nitrogen is fastest) and tumor/host variables. Local factors that slow down the freezing process are high tissue vascularity, density (bone is harder to freeze than soft tissue), and large volume.

The number of freezes applied per treatment session is important. Most small benign tumors are treated twice at the same sitting (freeze-thaw, freeze-thaw), while malignant, vascular, dense, or large tumors are generally treated three times. Little advantage in cell kill is gained by more than three freezes to the same volume of tissue.

A generally lethal temperature for most mammalian cells is stated to be −20°C. This minimum temperature is desired throughout the tumor. Colder temperatures are desirable when possible. The time that the tissue is maintained at a given temperature is not a crucial factor in cell death.

GENERAL TECHNIQUE[1,3]

Treatment of small externally accessible tumors (e.g., skin and anus) may be done under local anesthesia only. Treatment of other tumor sites may require sedation (eyelid) or general anesthesia (oral cavity) for adequate restraint of the patient.

A minimal clip of surrounding hair is performed. The tumor is then biopsied and debulked of redundant and easily removed tissue. Exposed hemorrhagic tumor tissue may be coagulated with silver nitrate or a suture ligature applied to the base of pedunculated lesions (Figure 15-1). Sterile placement of this ligature is not necessary, as it will slough with the frozen tumor tissue. The ligature provides the additional advantage of providing a "tourniquet" to decrease rewarming by the host. Debulking can be performed, as it will decrease the freezing time and allow colder temperatures to reach the tumor margins (Figure 15-2). Freezing large tumor volumes to depth has no advantage to freezing the margins to depth after debulking.

Once the tumor has been biopsied, it can be frozen by probe or spray. Probe freezing is adequate for smaller lesions that are more or less spherical, since the ensuing ice ball is spherical. The ratio of probe size to tumor should approach 1. This facilitates a faster freeze of tumor tissue. Warm probes should be applied to a warm/moist tumor surface and freezing is begun. A bond of the probe to the tumor (cryoadhesion) will rapidly ensue, and this bond is maintained throughout the freeze (30 seconds to a few minutes). A probe will not readily adhere to dry, intact epithelium. If the probe
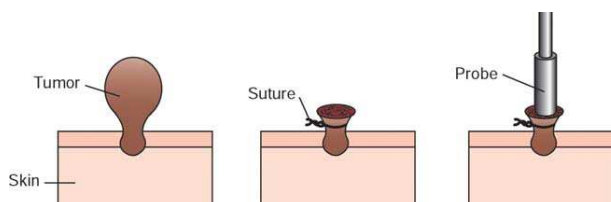
Figure 15-1

A pedunculated tumor is first removed at the skin surface for biopsy purposes and to reduce the tumor volume for freezing with a probe.
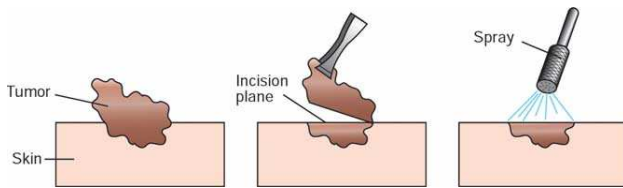
Figure 15-2

Exfoliative tumor volume is reduced with a scalpel prior to spray freezing.

prematurely detaches from the tumor, both probe and tumor should be allowed to thaw and the process begun again. Once the desired depth of freezing has been achieved, active freezing is stopped. The probe may take several minutes to detach and should not be forcibly detached, as undesirable tissue injury may occur. Once the tumor surface is moist again, the tumor is refrozen.

The author prefers spray freezing with liquid nitrogen to probe freezing, since it is faster, more lethal to depth, and more easily applied to nonspherical lesions. Various-size spray orifices are provided with most machines. Practice is required to learn how to control the fine spray of N_2 to just cover the tumor or desired tissue and prevent run-off. Careful observation and palpation of surrounding tissue are necessary to avoid inadvertent freezing of normal tissue. Insulation (styrofoam cups, vaseline, etc.) is only rarely employed and, in fact, may be dangerous, since it may act as a "cryoprobe" when frozen and cover up frozen tissue below it. Sufficient practice with spraying will virtually eliminate the need for insulators.

Regardless of probe or spray freezing, adequate margins of normal tissue should be frozen outside the lesion. This may be several millimeters for benign lesions or as much as 1 cm for malignant lesions. Although thermocouples are ideal for monitoring temperatures in tissue, visual observation and palpation of the "ice ball" are suggestive. The ice ball is anything below 0°C, and since mammalian cells are only reliably killed at –20°C, the entire ice ball will not die. Approximately 70% to 80% of the frozen tissue will slough. If you guess correctly and freeze the entire tumor to –20°C, the lesion should not recur. If you guess wrong, tumor recurrence is likely.

EXPECTED TISSUE RESPONSE

Immediately after freezing, the patient may experience mild discomfort. This rarely requires major pain relief and is absent within 12 hours. The frozen tissue will swell slightly and turn a darker color due to necrosis

and collapse of blood vessels. If exposed tissue is present (ulcerated tumor or biopsy surface), it may ooze a small amount of serum or blood. This usually forms a dry scab within several days. Mucous membrane sites (oral, eyelid, anus) may have a moist discharge for up to a week or more.

Even though the tissue may *look* infected by standard criteria, antibiotics are rarely necessary. In essence, the frozen and dead tissue acts like a biologic Band-Aid, during which time the body is producing bacteria-resistant granulation tissue at the deep junction of dead-to-viable cells.

Within 10 to 21 days, the scab will fall off, exposing a pink bed of epithelium (small lesions) or a reddish bed of granulation tissue that will contract or epithelialize (leaving a small hairless area).

Superficial layers of frozen necrotic bone will generally be adherent and may require debriding 2 or 3 months postfreezing. Hair regrowth may be white or gray on the periphery of the lesion due to death of melanocytes and preservation of hair follicles.

Owners are instructed to leave the area alone, afford minimal general hygiene, and keep the pet from licking or rubbing the area. Most animals leave the treatment site alone, presumably because of death of sensory nerves. A routine recheck is requested at 2 to 3 weeks, and subsequent rechecks are determined by the histologic tumor type and its biologic behavior.

SPECIFIC TUMOR SITES

Although many tumor types and sites have been frozen, experience has suggested four specific sites that may be treated by cryosurgery with predictably successful results (eyelid, perianal, oral, and skin). As a general rule, large, malignant cancers should be frozen only as a last resort because cryosurgery does not afford evidence of surgical margins for pathologic review, and further appropriate treatment must be delayed until recurrence. Additionally, large lesions (greater than 2.5 cm) will have a protracted healing time when compared to surgery. With this in mind, tumor types treated in the following sites are generally benign (or only locally invasive) and small (<2.5 cm).

Eyelid

Benign tumors of the eyelid[4–6] (meibomian gland adenoma, papilloma, etc.) are common in older dogs. Although not life threatening, they may cause problems from local irritation or corneal ulceration. Surgical excision and reconstruction of the eyelid can be performed but often require general anesthesia and longer treatment time than cryosurgery.

Most patients require light sedation or anesthesia prior to treatment. Local anesthesia at the base of the

tumor is usually performed. A chalazion forcep is then applied around the tumor and eyelid, which allows the lid to be held steady and removes it from contact with the globe. Additionally, the forcep acts as a tourniquet, reducing blood flow to the tumor and thus speeding the freezing process.

The excised portion of the tumor is then submitted for biopsy purposes. Silver nitrate should be used with caution to prevent exposure of the conjunctiva to irritation. A cryoprobe (small lesions) or a cryospray is then applied to the tumor and a few millimeters of surrounding eyelid. This is generally a full-thickness freeze. Any length of eyelid may be frozen with good function upon healing.

The eyelid is unique in its properties to maintain near-normal cosmetics and function after freezing. The eyelid is supported by a circumferential array of connective tissue called the tarsal plate. After freezing, the tarsal plate remains intact to allow epithelialization, while adnexal structures (eyelashes, sebaceous glands, and tumor) will die and be removed. Most cryosurgery sites are covered with pink epithelium by 2 weeks and have a minimal recession of the eyelid margin. The area will slowly repigment over 3 to 6 months, whereas cilia will not regrow.

Recurrence rates are less than 5%, which is at least as good as surgery with less anesthesia and surgery time and comparable cosmetics. Large, malignant tumors or tumors fixed to the orbit should generally be excised, including enucleation and orbitectomy if indicated.

Perianal

Perianal tumors[7] are most commonly seen in the older intact male dog (see Chapter 21, Section H) and are usually perianal adenomas. Cryosurgery can be an effective treatment for isolated and defined lesions that do not encompass more than 90° of the anal circumference.

Since most dogs are under general anesthesia for castration, freezing can be performed at the same time. The superficial aspect of the tumor or a wedge of tissue is removed with a blade, and a suture at the base (ligature or mattress) is applied for homeostasis. The tumor is then frozen with care to avoid extensively freezing the anal and rectal mucosa. No more than 50% of the anal circumference should be frozen to avoid anal stenosis resulting from fibrosis. Multiple lesions may be frozen as long as they are separated by normal unfrozen tissue.

The resulting eschar may be moister than most haired-skin cryosurgery sites but still does not require antibiotics. Elizabethan collars may be utilized if perineal licking is severe.

Local recurrence rates when combined with castration have been less than 5%. The advantages of cryosurgery over blade excision are speed, economy,

and lack of infection. Deeply invasive, 360°, and malignant cancers should generally not be frozen.

Oral

Cancer of the oral cavity[8] may invade bone. Eradication of tumor in bone can be accomplished with a variety of methods, including cryosurgery. Freezing is generally applicable to low-grade tumors that are adherent or minimally invasive into one cortex. Full-thickness freezing of the maxilla or mandible will usually result in an oronasal fistula or fracture, respectively. Freezing of normal bone will significantly reduce its breaking strength for one year or more.[9] If the bone can be protected from stress and infection (impossible in the mouth), the full-thickness frozen bone can act like an autograft and become revascularized and viable.

The advantage that cryosurgery has in minimally invasive bone lesions is destruction of tumor cells within the bone and maintenance of a bony framework for preservation of function. Tumors with extensive fixation to bone or invasion should generally be surgically removed. Freezing of pharyngeal or tonsillar lesions may result in life-threatening edema and swelling.

Under general anesthesia, regional radiographs will help determine the extent of bony invasion. Tumors adherent to underlying bone without evidence of radiographic bone involvement must still be considered invasive into the underlying bone. If the extent of bone invasion does not preclude freezing, the tumor is aggressively debulked and biopsied. The underlying bone is frozen three times with at least a 5-mm margin. Depth of freezing can only be determined by placing a thermocouple into underlying bone through a small drill hole. Depth of freezing in bone will not be as easily accomplished as in soft tissues.

After freezing, the frozen soft tissue will slough off rapidly due to abrasion by food and the tongue. A superficial area of dead bone 2 to 3 mm in depth will become necrotic. This will often form a sequestrum that may take several months to fall off or can be electively removed when it becomes loose to the touch. Once the sequestrum is removed, healing progresses rapidly.

A rare reported complication is nitrogen embolization.[10] This problem is induced when nitrogen is sprayed under pressure into exposed cancellous bone and expands intravenously. These nitrogen emboli then travel to the right atria and cause cardiovascular compromise and death. Theoretically, cardiocentesis and removal of nitrogen could be beneficial.

Control rates for oral malignancy in the published literature are generally poor. This is largely due to poor case selection (malignant, advanced stage, large) rather than inherent resistance to cryosurgery.

Skin

Benign skin tumors,[11] particularly in the dog, are perhaps the most common tumor condition in pet animals. Most of these tumors are not life threatening, and treatment is for cosmetic, inflammatory, and, occasionally, functional reasons. Freezing these tumors is fast, easy, safe, and economical.

A small area around the tumor is clipped of hair to prevent it from matting on the ensuing eschar. The skin and subcutaneous tissue is then infiltrated with a local anesthetic. The top of the tumor or a wedge of tissue is removed for biopsy purposes, and the tumor is frozen twice.

Healing may produce white hair on the periphery of the frozen area and small areas of hairless epithelium in the center. Most cryosurgery sites are cosmetically normal.

Large mast cell tumors should not be frozen, as rapid degranulation may result in a release of histamine, heparin, and other vasoactive substances that may induce hypotensive shock. Small mast cell tumors (<1 cm) may be safely frozen but may have a marked local response.

DISADVANTAGES OF CRYOSURGERY

Several factors make cryosurgery less desirable than conventional surgery:

1. *Equipment expense*. Initial equipment expense for cryosurgery instruments, thermocouples, and Dewar (if using liquid nitrogen) can run from $1000 to several thousand dollars. This investment can be made up in savings from anesthesia, surgical expenses (sterilization, suture, etc.), and time.
2. *Evaporation of N_2*. Depending on how many cases are treated per week, evaporation of N_2 can be an inconvenience. Since most cases are elective and benign, they can be stored up and treated every 2 to 3 weeks to coincide with delivery of N_2.
3. *Aesthetics of necrotic tissue*. Cryosurgery is not as "clean" as blade excision, but if owners are advised in advance of expected responses and the advantages of cryosurgery, few owner complaints will arise.
4. *False hopes for cryosurgery*. Contrary to some published reports, a systemic immunologic response to released or altered tumor antigens killed *in vivo* has not been clinically documented. Cryosurgery is also not a panacea for untreatable conditions. If a tumor could not be excised and expected to granulate or epithelialize (e.g., tumors more than one third the circumference of a limb or tail), then it will not happen after freezing.
5. *Lack of surgical margins*. Because the deeper levels of tumor invasion are left *in situ*, the margins of tumor destruction cannot be determined. For most

conditions treated with cryosurgery (small, benign, and not life threatening), this is not crucial but may allow an undesirable time lapse between incomplete treatment and recurrence of malignancies.

ADVANTAGES OF CRYOSURGERY

1. *Speed*. Most lesions can be frozen faster than they can be surgically excised.
2. *Expense per treatment*. Although initial equipment expense can be substantial, cost per treatment for consumables (liquid nitrogen or nitrous oxide) is usually less than $5.
3. *Ease of treatment*. Once some experience has been gained, cryosurgery will be considered easier than surgical excision for most surgeons.
4. *Safety*. Because of the ability to avoid general anesthesia in many cases, the usual risk of general anesthesia is avoided.

TUMORS, CONDITIONS, OR SITES THAT SHOULD NOT BE FROZEN

1. *Osteosarcoma of long bones*. Although local tumor control may be achieved, secondary fracture through further weakened bone will invariably occur when full-thickness freezing of a long bone is performed.[9]
2. *Intranasal tumors*. Aggressive freezing of intranasal tumors will frequently result in serious loss of palatine bone and large oronasal fistulas.[12]
3. *Circumferential anus*. The anus (or any body orifice) should not be frozen 360° for fear of inducing a fibrotic stricture of the lumen.[7]
4. *Large mast cell tumors*. Rapid degranulation of killed mast cells may lead to profound hypotensive shock.
5. *Large aggressive tumors*. Large aggressive tumors with life-threatening potential are better treated surgically when possible.

In spite of a stable and limited role for cryosurgery in veterinary medicine, the use of cryosurgery in human medicine continues to expand. Cancer of the lung, liver, kidney, prostate, and bone may benefit from cryosurgery in carefully selected settings.[13-19] The possible immune stimulus after cryosurgery continues to be explored.[20]

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SECTION B
Hyperthermia

Jean M. Poulson

INTRODUCTION

Hyperthermia is the elevation of tissue temperature above normal physiological levels. Thus, the term *hyperthermia* encompasses a wide range of temperatures and modalities. Cautery has been used throughout medical history to treat local disease, including cancer. An Egyptian papyrus describes treating a breast cancer patient with hyperthermia more than 5000 years ago.[1] Today, radiofrequency thermal ablation using a temperature of 60°C is being used to treat hepatic, renal, and lung tumors.[2-4] In the 1800s, reports of tumor regression in cancer patients who had prolonged high fevers led William B. Coley to investigate the use of extracted bacterial toxins to induce elevated body temperatures in patients with malignancies.[5] Coley's results were positive, possibly due to a combination of fever-range hyperthermia and nonspecific stimulation of the immune response. Presently, whole body fever-range hyperthermia (39.5°C to 40°C) is being investigated as a method to enhance immunological function for use as an adjuvant with cancer immunotherapies such as vaccines.[6,7]

Hyperthermia in the range of 40°C to 45°C as a local and regional therapy has been studied intensively during the past century. An increasing number of positive randomized clinical trials demonstrate that adding local hyperthermia to radiation therapy results in a better outcome than radiation therapy alone. Evidence suggests that there are important biologic effects of hyperthermia that occur in the range of 39°C to 42°C, including inhibition of radiation damage repair, tumor reoxygenation, improved tumor blood flow, induction of the heat shock response, and immunological stimulation.[8-10] Local and regional hyperthermia protocols in this lower temperature range might improve tumor response to radiation and chemotherapy by exploiting these effects. This lower temperature range is also proving effective for strategies involving novel drug delivery and regulation of gene therapy in solid tumors.

BIOLOGY

Hyperthermia causes a number of direct effects on both cells and tissues. Heat alone at temperatures exceeding 41.5°C causes cytotoxicity that is both time and temperature dependent.[11] Sensitivity to heat is increased in acidotic cells[12,13] and in cells that are in the S-phase of the cell cycle. Hypoxic cells are at least as equally sensitive to heat as aerobic cells.[14] Since cells in S-phase are less sensitive to radiation, and hypoxic cells may be two to three times less sensitive to radiation than well-oxygenated cells, hyperthermia has been combined with radiation therapy to take advantage of these complementary cytotoxic effects. Hyperthermia also sensitizes cells to radiation by inhibiting the repair of radiation-induced DNA damage. Hyperthermia effects on tumor physiology also improve radiation therapy.

Hyperthermia has been shown to improve oxygenation in human and canine soft tissue sarcomas,[15,16] which should further increase radiation sensitivity. This improvement in oxygenation appears to decrease at temperatures above 44°C, suggesting that there may be substantial benefits at lower temperatures more easily achieved in the clinic. In a randomized phase III clinical trial of hyperthermia combined with radiation therapy in dogs with soft tissue sarcomas, local tumor control was improved with a high thermal dose compared with a low thermal dose.[17] In addition, duration of heating was inversely related to outcome. This effect suggests that factors other than thermal dose, such as physiologic alterations caused by heating, may have some influence on tumor response to hyperthermia combined with radiation therapy.

HYPERTHERMIA DELIVERY

A variety of methods and devices are used clinically to induce hyperthermia in tissues. Noninvasive methods using radiofrequency currents, microwaves, or ultrasound are the most common. Heating of solid tumors is typically nonuniform, due in part to the heating devices available and in part to nonuniform distribution of blood flow in the tumor. The lack of uniformity of heating makes measurement of temperatures during hyperthermia critical but challenging. Presently, invasive thermometry is the method most commonly used. Catheters are placed into the tumor, and interstitial temperatures are measured at regular intervals along these paths. While this method still often leaves a large volume of the tumor unmapped, it can be used to prospectively deliver a prescribed hyperthermia dose.[18] Noninvasive thermometry methods using magnetic resonance imaging (MRI) are being developed to overcome the limitations of this invasive system.[19–21] Noninvasive thermometry will allow temperature measurement throughout the entire tumor volume, and allow for optimization of tumor heating by real-time adjustment of heating devices during treatment. Further development of clinical hyperthermia equipment, resulting in wider availability, and the clinical implementation of noninvasive thermometry will facilitate more common use of hyperthermia in the management of cancer in both humans and animals.

DRUG DELIVERY

Hyperthermia induces changes in microvessel pore size that can lead to increased accumulation of liposomes[22] and increased delivery and extravasation of liposomally encapsulated drugs.[23,24] Increased extravasation of liposomes with hyperthermia appears to be preferential to tumors[23] and can be further exploited by using temperature-sensitive liposomes to deliver the drug as a burst release.[25] A phase I trial of temperature-sensitive liposomes in dogs with spontaneous tumors demonstrated the feasibility of this approach.[26] Delivery of macromolecular therapeutic agents, such as monoclonal antibodies,[27] or thermally responsive drug-carrying polymers[28] can also be directed and enhanced with local hyperthermia.

GENE THERAPY

Hyperthermia represents a unique approach to the regulation of gene expression for gene therapy. Heat shock induces a specific set of genes, including the heat shock protein (HSP) 70 promoter. Adenovirus vectors containing therapeutic genes such as interleukin-12 (IL-12) under the control of the HSP70 promoter have been developed for intratumoral injection followed by local hyperthermia. This strategy was developed to achieve high intratumoral concentrations of IL-12 while avoiding the unacceptable toxicity associated with systemic administration of IL-12. Preclinical studies in mice using this vector yielded tumor growth delay superior to heat alone or gene therapy alone.[29] The addition of radiation therapy to thermoimmunogene therapy further enhanced tumor growth delay.[30] A phase I study of thermoimmunogene therapy in pet cats with vaccine-associated sarcomas showed that this approach is feasible in the clinical setting and warrants further investigation.[31]

CLINICAL TRIALS

There are ten positive, randomized clinical trials demonstrating that when hyperthermia is added to radiation therapy of solid tumors the outcome is better than with radiation therapy alone. Seven of these were human clinical trials[32–38] and three were clinical trials in pet dogs with spontaneous tumors.[39–41] Two trials in which a prescribed thermal dose was prospectively applied, one in humans,[42] and one in dogs,[17] demonstrated a positive association between increased thermal dose and local tumor control. These results illustrate not only the benefit of hyperthermia when combined with radiation therapy, but also the importance of temperature measurement during clinical hyperthermia. This adds impetus to the work being done to develop noninvasive magnetic resonance thermometry for clinical use.

CONCLUSION

New approaches to the use of hyperthermia, ongoing advances in clinical equipment and temperature measurement, and an increasing understanding of the

biologic and physiologic effects of heat are broadening the potential for its clinical use in cancer management. Positive thermal dose escalation trials are promising. The ideal strategy for clinical hyperthermia treatment, including thermal dose, fractionation, and time and temperature goals, has yet to be identified. Active research programs are continuing to develop technology to improve temperature distribution and thermometry methods for clinical use, and to define the relationships between thermal parameters, physiologic effects, and response of solid tumors to hyperthermia in combination with radiation therapy or chemotherapy. Clinical studies in animal cancer patients will help improve the therapeutic benefit from hyperthermia for both humans and animals.

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SECTION C
Photodynamic Therapy

Michael D. Lucroy

INTRODUCTION

The practice of using sunlight to treat disease is ancient, but modern refinements have allowed the interactions between light and drugs to evolve into a highly effective cancer treatment called photodynamic therapy (PDT).[1] PDT relies on the simultaneous presence of a photosensitizer, visible light, and molecular oxygen to cause a photochemical reaction capable of selectively destroying malignant tissue (Figure 15-3). PDT causes tumor cell death through a combination of apoptosis, ischemic necrosis, and actions of immune effector cells. Although *in vitro* resistance to PDT has been observed in cells with defective apoptotic pathways,[2] *in vivo* tumor resistance to PDT does not seem to occur.[3] Because PDT is typically effective with a single treatment, spares normal tissues, and is associated with few systemic adverse effects, it offers many potential advantages for veterinary cancer patients over systemic chemotherapy, ionizing radiation therapy, and aggressive surgery in critical areas, such as the oropharynx or lower urinary tract.[4]

Although initially studied as a single modality, PDT may also be useful when combined with other cancer treatments. Early studies show that a combination of PDT and low-dose cisplatin increases efficacy both *in vitro* and in murine models,[5,6] and similar synergy has been observed when doxorubicin is combined with PDT.[7,8] PDT also upregulates COX-2, but not COX-1 expression, and the combination of COX-2 inhibitors with PDT increases the efficacy of PDT in rodent tumors.[9,10] The observation that the tumor-localizing porphyrin photosensitizers are effective radiosensitizers provides rationale for combining PDT with external beam radiation therapy.[11,12]

Photosensitizers

To begin the process of PDT, the patient is given a light-reactive drug called a photosensitizer, which preferentially
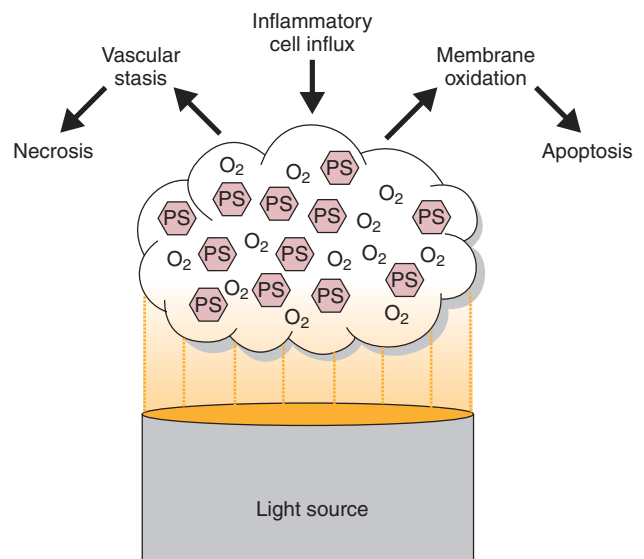
Figure 15-3

Conceptualization of how photodynamic therapy works. Light of the appropriate activating wavelength interacts with a photosensitizer (PS) selectively retained within the tumor. The excited PS interacts with molecular oxygen (O_2), creating reactive oxygen species that are responsible for causing vascular stasis and necrosis, membrane damage, and apoptosis, and starting a signaling cascade resulting in an influx of inflammatory cells.

localizes in neoplastic tissue. Tumor characteristics, drug delivery vehicle, and photosensitizer properties may all play a role in tumor localization; however, the precise mechanisms are largely unknown. Tumor factors that may contribute to the selective retention of photosensitizers include a leaky vasculature, large interstitial space, acidic pH, high lipid content, abundance of newly synthesized collagen, presence of macrophages, and increased numbers of low-density lipoprotein receptors.[13,14] Low tumor pH, compared to normal surrounding tissue, increases lipophilicity, thereby increasing cellular uptake of certain photosensitizers.[15,16] Decreasing pH may also contribute to more cell membrane damage by affecting where the photosensitizer localizes within lipid membranes.[17] Intratumoral lipid binds lipophilic photosensitizers, and newly formed collagen has an affinity for porphyrin photosensitizers.[18] Tissue macrophages take up large amounts of certain photosensitizers and are potentially important in delivering drug to the tumor.[19] Increasing photosensitizer hydrophobicity increases tumor localization, which may be further enhanced when coupled with liposome delivery systems.[20,21] Associating photosensitizers with low-density lipoproteins causes their preferential release to tumor cells.[22] At present, the relative significance of each of these factors is unknown and various delivery systems are under investigation.[23,24]

An alternative to administering a preformed photosensitizer is the concept of endogenous photosensitization, where the patient is given a pro-photosensitizer that is preferentially metabolized by malignant cells into a photoactive compound. The best characterized agent for endogenous photosensitization is 5-aminolevulinic acid (ALA), which is preferentially metabolized by malignant epithelial cells into protoporphyrin IX (PpIX), a highly efficient photosensitizer.[25] Because PpIX accumulates slowly in normal epithelium after ALA administration, and is virtually undetectable in mesenchymal cells, ALA-based PDT is highly selective for carcinomas.[26] In addition to intravenous administration, ALA may also be applied topically or given orally. Considerable efforts have been made in developing imaging systems to detect ALA-induced PpIX within malignant or premalignant epithelial tissue as a noninvasive method of photodiagnosis.[27–31]

Many photosensitizers have been investigated for PDT in veterinary medicine, including hematoporphryin derivative (HpD), porfimer sodium (Photofrin), 5-aminolevulinic acid (ALA), 5-ethylamino-9-diethylaminobenzo[*a*]phenothiazinium chloride (EtNBS), meta-tetrahydroxyphenylchlorin (mTHPC), 2-[1-hexyloxyethyl]-2-divinyl pyropheophorbide-a (HPPH), tin etiopurpurin (SnET2), aluminum phthalocyanine tetrasulfonate (AlPcS$_4$), and zinc phthalocyanine tetrasulfonate (ZnPcS$_4$).[32–46] The large number of photosensitizers under consideration is an indication that the ideal photosensitizer has not been identified. Useful properties of a good photosensitizer for veterinary applications include low systemic toxicity, preferential uptake and retention by tumor tissue, good photosensitization efficiency with wavelengths of light easily transmitted through tissue (>600 nm), and rapid clearance from the skin to minimize cutaneous photosensitivity.[4] A major concern with PDT in humans is prolonged cutaneous photosensitization. For example, Photofrin, the first photosensitizer approved by the Food and Drug Administration (FDA) for human use, can cause severe cutaneous photosensitization reactions in people up to 8 weeks after administration.[47] Cutaneous photosensitization appears to be an uncommon problem in dogs and cats treated with PDT, which may reflect the limited use of Photofrin in these species, differences in photosensitizer distribution compared to humans, or differences in the skin and adnexa between species. Currently there are no FDA-approved photosensitizers for veterinary use.

Light

Delivery Systems

The choice of photosensitizer dictates which wavelength of light is required for treatment because each drug has a particular peak activating wavelength, typically within the visible red spectrum. Light wavelength is directly proportional to tissue penetration, with light >630 nm (i.e., visible red light) being transmitted easily through tissues, and is therefore well suited for PDT.[48–51] Light is delivered to the target tissue through surface, interstitial, or intraoperative irradiation (Figure 15-4). During surface irradiation, laser light from an optical fiber terminating in a microlens is focused on the tumor. Optical fibers terminating in cylindrical diffusers are placed directly into tumors for interstitial irradiation. Special optical fibers, designed for use with flexible endoscopes, are used to irradiate esophageal and endobronchial cancers. Because these optical fibers are small, from 200 to 1000 μm in diameter, virtually any site within the body is accessible for fiber placement.

In ancient times, physicians relied on sunlight as their light source for light therapy. However, the development of the medical laser in the 1960s provided PDT researchers a tool capable of delivering tremendous amounts of monochromatic light. The dye laser, tunable over a wide range of visible wavelengths, was used for many early PDT studies. In response to the limitations of tunable dye lasers for clinical use, namely their physical size, expense, and requirement for specialized electrical and plumbing connections, several new light devices have been developed. These include portable, single-wavelength diode lasers, light-emitting diode (LED) arrays, and filtered lamp devices (Figure 15-5).
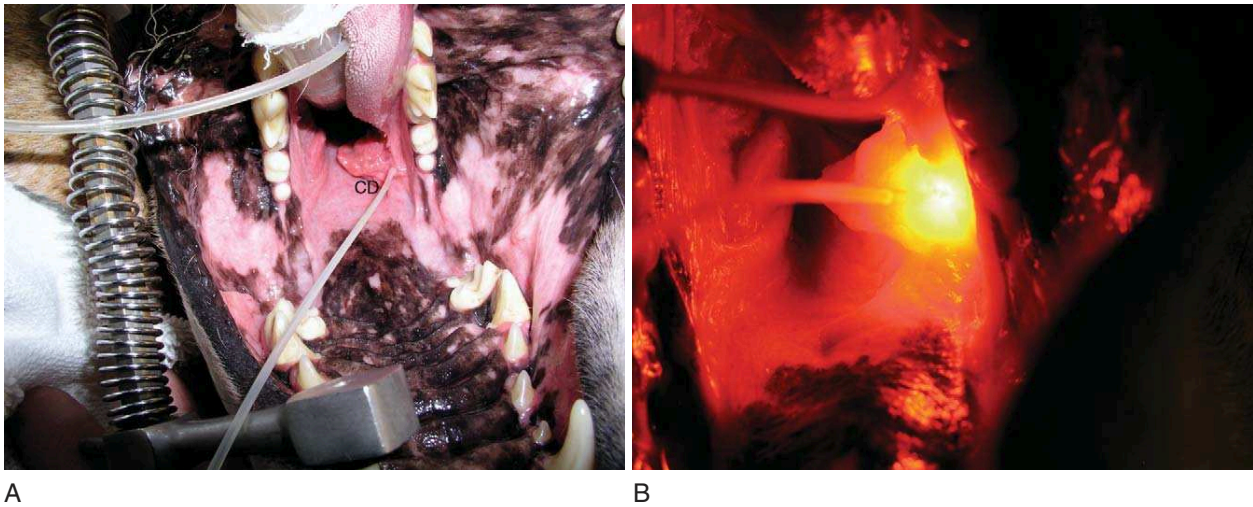
Figure 15-4

Photodynamic therapy of tonsillar squamous cell carcinoma in an anesthetized dog. An optical fiber terminated in a cylindrical diffuser (CD) is placed into the tumor (**A**), and then the visible red laser light launched through the fiber to activate the photosensitizer (**B**).

An LED array has been used to treat superficial squamous cell carcinoma (SCC) in cats, representing the first report of a clinically applicable nonlaser alternative for veterinary PDT.[52] A filtered lamp device, capable of approximating the spectral output range of a tunable dye laser, has more recently been used in veterinary medicine. The *in vitro* efficacy of PDT using the filtered lamp device was found to be equivalent to a laser. Preliminary clinical results from a small number of dogs and cats with superficial carcinomas treated with PDT using the filtered lamp were promising, with a median disease free interval >476 days.[53] Nonlaser light sources have two current disadvantages when compared to lasers. First, the nonlaser light sources may have limited power output, which result in longer treatment times, especially for large treatment areas. Second, it is difficult to launch nonlaser light into optical fibers, limiting their use with flexible endoscopy systems. However, improvements in design and engineering may overcome these limitations. Clear advantages of the nonlaser light sources for PDT over medical lasers include the decreased equipment cost and lack of laser-associated eye hazards. Further evaluation of these nonlaser light sources may reveal they are robust and cost-effective solutions for veterinary PDT; equipment expense has been a longstanding limiting factor in the acceptance of PDT in both human and veterinary medicine.[54]

Dosimetry

During PDT, light is applied to the tumor and a small area of surrounding normal tissue. The light dose, also

Figure 15-5

Photodynamic therapy of a dermal mast cell tumor on the foot of a dog using a nonlaser, filtered lamp light source. Note the difference in optical fiber bundle (OFB) diameter compared to the laser fiber shown in Figure 15-4.

known as fluence or energy density, is expressed as Joules per square centimeter. The power density refers to the rate of photon delivery and is expressed as watts per square centimeter. The power density is limited by the maximum power output from the light source and depends on the tumor surface area to be treated, as the required power output for PDT is calculated by

multiplying the surface area to be treated by the power density. With a given power output and fixed energy dose, as the treatment area increases, the treatment time becomes longer. The treatment time is calculated from the formula $J = W \div S$, where J is energy in Joules, W is power in Watts, and S is time in seconds. Therefore, treatment time in seconds = $W/cm^2 \div J/cm^2$. For most reported veterinary PDT applications, the energy dose is between 50 and 200 J/cm², with power densities ranging from 50 to 100 mW/cm².[4,55] Most treatments can be accomplished in less than 30 minutes, but large surfaces requiring multiple overlapping treatment spots can take substantially longer. Light dosimetry calculations are similar for interstitial or intraluminal PDT application, but they are based on the surface area of the light column emitted from the cylindrical diffuser.

Both laser power density and fluence affect the efficacy of PDT. At high power densities (>125 mW/cm²), tissue heating occurs, making it impossible to assess PDT effects independent of thermal effects, and may contribute to rapid photodegradation of the photosensitizer and depletion of oxygen.[56] Low power densities result in high rates of local tumor control and efficient tumor killing, but they require long light treatment times to deliver adequate energy.[57] Improved PDT efficacy with low power density may be the result of fewer microvascular effects, thereby preserving tumor blood flow, but could also be due to slower photochemical depletion of molecular oxygen within the treatment site.[58] Because PDT in veterinary medicine is done under general anesthesia, for both immobilization and analgesia,[59,60] the length of treatment time may be an important consideration in designing treatment protocols. There is evidence that using alternating light-dark intervals also increases PDT efficacy.[61,62] This is possibly a result of tumor reoxygenation occurring during the dark periods. The optimal light-dark interval for PDT remains to be determined and will likely vary with the photosensitizer used and tumor type treated. Although the current paradigm uses PDT as a single-session treatment, repeated PDT may improve treatment outcome.[63]

Photosensitizer Activation

As the photosensitizer absorbs photons, it changes its energy state to the excited singlet then triplet state.[64] As the photosensitizer returns to the ground state, it may transfer energy directly to proteins and other biological molecules in the vicinity (type I photosensitizer reaction) or to molecular oxygen (type II photosensitizer reaction). The resulting reactive oxygen species and free radicals can damage cellular structures and, if the damage overwhelms endogenous repair mechanisms, cause cellular death.[65,66] Intratumoral

oxygen required for PDT reactions is potentially limited by consumption during PDT and effects on tumor vasculature that diminish blood flow.[67]

The oxidative damage to cellular membranes can lead to apoptosis.[68,69] The photochemical reactions also cause vascular stasis, thrombosis, and ischemic necrosis.[70] Oxidized cellular membranes are potent pro-inflammatory signals, causing an initial influx of neutrophils.[71] The neutrophils may indiscriminately kill cancer cells, but they can also damage normal tissue. Macrophages arrive later and can process tumor antigens to present to the immune system and may react to heat shock proteins induced by PDT, representing another mechanism by which PDT affects local tumor control.[72] In murine tumor models, PDT induces tumor regression in a matter of days, and similar rapid effects have been observed in dog and cat tumors (Figure 15-6). Although *in vitro* resistance to PDT has been observed in cells with defective apoptotic pathways,[2] *in vivo* tumor resistance to PDT has not been observed.[3]

Clinical Applications

Squamous cell carcinoma

In veterinary medicine, PDT has been most commonly used in the treatment of squamous cell carcinoma (SCC). Most SCCs are superficial, localized, and do not metastasize until late in the course of the disease, making them well suited to treatment with PDT. An early description of chloro-aluminum sulfonated phthalocyanine-based PDT in 10 cats with superficial SCC or carcinoma *in situ* reported a 70% complete response rate, demonstrating the potential for PDT as a skin cancer treatment.[45] Although the photosensitizer dose was constant, the energy density and power density were varied between cats.

AlPcS₄-based PDT has been used to treat solar-induced SCC of the facial skin in cats. In one report, each cat was given 2 mg/kg AlPcS₄ intravenously, followed by laser irradiation 24 hours later.[44] Each lesion, and a 1 to 2 mm surrounding margin of normal tissue, was treated with 675 nm light (100 mW/cm²; 100 J/cm²) from an argon-pumped tunable dye laser focused on the tissue by an optical fiber fitted with a microlens. Eighteen cats with 19 lesions were treated. Complete responses were observed in 70% of the cats, with tumor progression-free intervals up to 18 months. Adverse effects associated with this PDT protocol included localized swelling, inappetence, cutaneous photosensitization, and hepatotoxicity.[44,73] Emesis associated with AlPcS₄ injection was also reported. This represented the first veterinary PDT report of a single tumor type, in a single species, treated with a fixed drug and light protocol.

Another report of AlPcS₄-based PDT for cutaneous SCC in cats demonstrated the effect of fluence on efficacy.[74]

Figure 15-6

Appearance of an acanthomatous epulis of the maxilla of a dog 24 hours after photodynamic therapy. Note the dark discoloration of the tumor and sharp line of demarcation between the tumor and surrounding normal-appearing gingiva, demonstrating the selectivity of photodynamic therapy-induced tissue damage.

Ten cats were given 1 mg/kg AlPcS$_4$ intravenously and treated with 675 nm light at either 100 or 200 J/cm^2. The maximum power density used was 200 mW/cm^2, which is sufficient to heat tissue, therefore making it is impossible to determine the PDT effects independently of hyperthermia. The overall remission duration was 151 days, and the overall response rate was 75%, making this protocol less effective than previous reports of PDT for SCC in cats. However, cutaneous photosensitization and hepatotoxicity were not reported in these 10 cats.

HPPH-based PDT has been used for the treatment of facial SCC in cats.[40] In this study, 61 cats were each given 0.3 mg/kg HPPH intravenously, followed by laser irradiation 24 hours later. All lesions were treated with 665 nm light (75 mW/cm^2; 100 J/cm^2) from an argon-pumped tunable dye laser. Light was focused on the tumor by an optical fiber fitted with a microlens, and the treatment area included a 5-mm margin of grossly normal tissue. For the smallest tumors (<1.5 cm in diameter), the complete response rate was 100%. For larger, invasive tumors (>1.5 cm in diameter), the complete response rate was only 18%. The 1-year local control rate for all tumors was 62%. The study design allowed a direct comparison of PDT efficacy to radiation therapy. In a similarly designed radiation therapy study, a fractionated course of external beam radiation therapy produced a comparable 60% 1-year progression free rate for all cats with SCC.[75]

Topical ALA cream has also been used to photosensitize facial SCC in cats for PDT.[52] In this study, tumors were irradiated with red light (635 nm) from an LED array. After a single treatment, the complete response rate was 85%. Seven of the 11 lesions undergoing a complete response subsequently recurred; the median disease-free interval was 21 weeks. Although the response duration was not as long as reported from other PDT studies, the clinical potential of nonlaser light sources for PDT was demonstrated. Furthermore, the topical delivery of ALA was not associated with the hematological and hepatic toxicities that may occur with intravenous administration of the drug in cats.[36]

In a study of the usefulness of a filtered lamp source for PDT, dogs and cats with superficial carcinomas were given 0.15 mg/kg HPPH, and the light treatment was performed 1 hour later.[53] All tumors achieved a complete response and had not recurred after 68 weeks of follow-up. Aside from demonstrating the utility of a nonlaser light source for PDT in veterinary medicine, this study suggests that the drug-light interval is another important variable that can influence the outcome of PDT.

Canine oral SCC has also been treated with PDT.[41] In one study, 11 dogs were given 0.3 mg/kg HPPH intravenously followed by 665 nm laser irradiation (mW/cm^2; J/cm^2) 48 hours later. Based on radiographic appearance, 7 of the 11 dogs treated had SCC invasion into underlying bone. Eight of 11 dogs were considered cured, with a disease-free interval greater than 17 months. Zinc phthalocyanine tetrasulfonate-based PDT has also been reported effective against oral SCC in a small number of dogs in a phase I clinical trial of this second-generation photosensitizer.[46] These results suggest that PDT has similar efficacy to segmental removal of portions of mandible or maxilla for the treatment of canine oral squamous cell carcinoma with good cosmetic and functional outcomes; however, direct comparisons accounting for variations in clinical tumor stage were lacking.

Lower urinary tract tumors

ALA-based PDT has been used to treat a small number of dogs with transitional cell carcinoma (TCC).[37] ALA is an ideal photosensitizer for PDT of the urinary tract, as the drug is metabolized to the active porphyrin only within the epithelium, obviating the risk of unintentional damage to the muscularis. Normal dogs treated with ALA-based PDT of the urinary bladder had only mild edema of the mucosa after treatment. Five dogs with TCC of the urinary bladder were treated with a single ALA-based PDT session, and all had a transient improvement in clinical signs, with tumor progression-free intervals ranging from 4 to 34 weeks. ALA-based PDT has also been used for managing prostatic carcinoma in a dog.[35]

Intranasal tumors

HPPH-based PDT has been used to treat a small number of dogs and a cat with intranasal tumors.[76] Three dogs and a cat with intranasal tumors were given 0.3 mg/kg HPPH by IV injection and treated with light 24 hours later. Using anatomic landmarks identified on pretreatment CT images, optical fibers terminated in cylindrical diffusers were advanced through the nostril to the level of the tumor to deliver 665 nm laser light. Facial swelling developed in all the animals, but was mild and self-limiting. The owners reported a decrease in sneezing and epistaxis soon after PDT. Although only two of the animals had survival times comparable to animals treated with radiation therapy; the results from the small case series suggests that PDT may be a viable treatment for intranasal tumors.

Soft tissue sarcomas

Intraoperative HPPH-based PDT has been used in a small number of dogs with hemangiopericytoma.[42] In this study, 16 dogs were given 0.3 mg/kg HPPH IV, and 48 hours later, the tumors were surgically removed and 665 nm light applied to the surgical bed. Several of the dogs had delayed wound healing or dehiscence, in some cases requiring skin graft revision surgery. The local recurrence rate was 56% in this small group, suggesting intraoperative PDT offers no advantage over surgery followed by radiation therapy for soft tissue sarcoma. The nature and severity of the local complications reported with intraoperative HPPH-based PDT suggests it may have limited applications. One dog with a body wall soft tissue sarcoma treated with CASPc-based PDT after failing surgery and radiation therapy died due to metastatic disease soon after PDT; however, at necropsy there was no evidence of cancer in the treatment site.[45] Additionally, $ZnPcS_4$-based PDT has been used to treat two dogs with large subcutaneous soft tissue sarcomas.[46] Both treatments produced only partial responses, in part due to the large size of the lesions to be treated. Although relatively few soft tissue sarcomas in dogs or cats have been treated with PDT, these early results suggest that mesenchymal tumors may not respond as favorably to PDT as epithelial tumors.

Other applications

The current uses for PDT in veterinary medicine are limited to the treatment of cancer. However, there are several other potential applications. PDT has been shown to eliminate feline leukemia virus and virus-infected cells from stored, whole blood.[77] PDT has also been used to kill various bacteria and fungi, which may portend its use for chronic skin and ear infections in dogs and cats.[78–81] PDT may also be useful for treating premalignant diseases such as actinic keratosis.[82–84] In people, PDT is now being used to inhibit the abnormal growth of blood vessels that causes adult-onset blindness in macular degeneration.[85,86] This suggests the possibility of developing similar PDT applications to treat vascular abnormalities, such as dermal hemangioma and hemangiosarcoma, in veterinary patients.

Although many types of tumors have responded favorably to PDT, aside from facial squamous cell carcinoma in cats, there are presently too few treated cases to determine what the utility of PDT for many tumors in veterinary medicine. PDT may not offer large advantages for small superficial tumors over traditional treatment, but it has great potential for intraluminal lesions such as the urinary and respiratory tract. Because PDT is seemingly well tolerated by most animals, it may represent a safe, cost-effective treatment alternative for many solid tumors.

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16

Supportive Care for the Cancer Patient

SECTION A
Management of Chronic Cancer Pain

B. Duncan X. Lascelles

In 1978, Yoxall[1] stated: "It is surprising, for instance, how much a dog's quality of life, observed by the owner, may be improved by the administration of a simple analgesic if the dog is suffering from a tumor, which although painless on palpation, may be causing considerable chronic pain." Despite this statement, and the fact that obvious pain associated with specific tumors such as osteosarcoma has been emphasized for a long time as a diagnostic criterion, there is an almost complete absence of controlled studies that specifically investigate the occurrence of cancer pain in companion animals or the alleviation of pain in animals suffering from cancer.[2-5] Given the relative lack of animal clinical studies, the information in this chapter cannot be based on peer-reviewed investigations. Rather, it is a combination of the author's experience and the experience of others who are heavily involved in the treatment of cancer patients. It is also based on considered extrapolations from human medicine and from veterinary research in other chronically painful conditions, such as osteoarthritis.

This section of the chapter deals with the treatment of chronic cancer pain in dogs and cats. The control of acute perioperative pain in cancer patients is also very important, and readers are encouraged to refer to appropriate texts for information on perioperative pain control.[6,7]

HOW COMMON IS CANCER PAIN IN DOGS AND CATS?

The prevalence of cancer in the pet population is difficult to estimate accurately. One study looked at the cause of death in 2000 dogs presented for postmortem examination. It revealed that 45% of dogs living to 10 years or older died of cancer and, with no age adjustment, 23% of patients presented for necropsy had died of cancer.[8] In a survey, 2000 respondents stated that cancer was a leading cause of death in both dogs (47%) and cats (32%).[9] These figures suggest that cancer has a significant prevalence within the pet population.

HOW PAINFUL IS CANCER IN ANIMALS?

Not all tumors are painful, and the amount of pain is likely to vary considerably from one animal to another, even with similar tumor types. The author's experience and the experience of others would suggest that, using a conservative estimate, 30% of tumors in dogs and cats are associated with significant pain at the time of diagnosis. Tumors most likely to be associated with pain include those at the following sites: oral cavity, bone, urogenital tract, eyes, nose, nerve roots, gastrointestinal tract, and skin (Table 16-1). The 30% estimate is likely conservative since pain is experienced by 20% to 50% of human patients when the lesion is diagnosed, by nearly half undergoing active treatment, and by up to 90% of patients with far advanced or terminal cancer. In humans, an overall average of about 70% of patients with advanced cancer suffer pain.[10,11]

In addition to pain caused by the tumor itself, pain in cancer patients can also be caused by chemotherapy, radiation therapy or surgery (perioperative pain, postoperative pain, and conditions such as "phantom limb" associated with some amputations), and concurrent noncancerous disease, most notably osteoarthritis.

GENERAL APPROACH TO CANCER PAIN MANAGEMENT

The incidence of pain in animals due to cancer is difficult to estimate, as is the effectiveness of analgesic therapy. Surveys have found that significant numbers of animals

TABLE 16-1	List of Tumors Most Likely to Be Associated with Pain
Tumors Category	**Notes**
Tumors involving bone	Primary bone tumors and metastasis to bone are usually painful.
Central nervous system tumors	Extradural tumors are often associated with pain; however, tumors originating from within the neural tissue are not associated with pain until later on in the course of the disease. In humans with brain tumors, 60% to 90% suffer from headaches.
Gastrointestinal	Especially esophagus, stomach, colon, and rectum. Colonic and rectal pain is often manifested as perineal discomfort.
Inflammatory mammary carcinoma	Dogs with this form of mammary cancer appear to exhibit obvious signs of pain.
Genitourinary tract tumors	Stretching of the renal capsule appears to produce significant pain. Bladder tumors appear to be predictably associated with pain. Tumors of the distal genitourinary tract are often manifested as perineal pain.
Prostate	Prostatic tumors appear to be particularly painful, especially if local metastasis to bone is present.
Oral and pharyngeal tumors	Soft tissue tumors projecting from the gingival surface appear to be relatively nonpainful. Tumors involving bone appear to be significantly more painful. Soft tissue tumors of the pharynx and caudal oral cavity are particularly painful.
Intranasal tumors	Pain probably results both from the destruction of turbinates and from the destruction of bone of the nasal cavity.
Invasive cutaneous tumors	Especially those that are ulcerative.
Disseminated intrathoracic and intra-abdominal tumors, (e.g., mesothelioma, malignant histiocytosis)	The signs associated with such tumors are particularly vague; however, intracavitary analgesia (such as intra-abdominal local anaesthetic) can markedly improve the animals demeanor, and thus it appears that disseminated neoplasia of these cavities is associated with significant pain.
Pain following surgical removal of a tumor	Pain well beyond the postoperative period occurs in some animals that have undergone surgery and is probably neuropathic. Most often pain is associated with tumor recurrence. True phantom pain (such as phantom limb pain) that is not associated with tumor recurrence does appear to exist in animals.

in the perioperative setting were not receiving analgesic drugs.[12-18] It is likely that the use of analgesics for cancer pain is even less. Glucocorticoids do provide some analgesia, and their use may be more widespread,[14] but the specific treatment of cancer pain in animals is still likely to be suboptimal.

Suboptimal treatment of cancer pain in animals probably results from a number of factors:
- Lack of appreciation that many cancers are associated with significant pain
- Inability to assess pain in cancer patients
- Lack of knowledge of drugs, drug therapy, and other pain-relieving techniques
- Lack of communication with clients and lack of involvement of clients in the assessment and treatment phases
- Under-use of nursing staff for assessment and reevaluation of pain in cancer patients

These barriers can be effectively overcome by the following:
1. Educating and training veterinarians about the importance of alleviating pain, assessment of pain,
available drugs and potential complications, and interventional techniques
2. Educating the client about realistic expectations surrounding pain control
3. Ensuring that both the veterinarian and the owner recognize that owner involvement in evaluating the pet and providing feedback on therapy is crucial to success
4. Continually reassessing the pet's pain throughout the course of therapy

Although drug treatment is the mainstay of cancer pain treatment, effective cancer pain treatment often involves a combination of drug therapy, nondrug therapies, and good communication between all parties involved. A basic approach to cancer pain management can be summarized as follows:
1. **A**ssess the pain. Ask for the owner's perceptions of the pain present or of any compromise of the animal's quality of life.
2. **B**elieve the owner. The owner sees the pet every day in its own environment and knows when alterations in behavior occur. Owners can rarely

suggest diagnoses, but they do know when something is wrong and when their pet is in pain, just as a mother knows when something is wrong with her child.

3. **C**hoose appropriate therapy depending on the stage of the disease. Anything other than mild pain should be treated with more than one class of analgesic, or an analgesic drug combined with nondrug adjunctive therapy. Be aware of potential drug interactions.

4. **D**eliver the therapy in a logical coordinated manner and carefully explain to the owner any possible side effects.

5. **E**mpower the clients to participate actively in their pet's treatment; ask for feedback and updates on how the therapy is working.

THE IMPORTANCE OF ALLEVIATING PAIN

Pain can induce a stress response in patients.[19] A prolonged stress response can decrease the rate of healing. In addition, the stress response can have adverse effects on the cardiovascular and pulmonary systems, fluid homeostasis, and gastrointestinal tract function.[19]

The alleviation of pain is important not only from a physiological and biological standpoint, but also from an ethical point of view.[20] Veterinarians have an ethical obligation to treat pain in the animals under their care. Insufficient treatment of pain is probably a result of lack of adequate knowledge and not a lack of concern. Pain is only one factor that negatively impacts welfare. To help in an evaluation of welfare, five freedoms, initially proposed by the Brambell Committee in reference to farmed animals,[21] have been suggested. They may equally be applied in the context of companion animals.[20] They are as follows:

- Freedom from hunger and thirst
- Freedom from physical and thermal discomfort
- Freedom from pain, injury, and disease
- Freedom to express normal behavior
- Freedom from fear and distress

For each freedom, there should be a consideration of the severity, incidence, and duration. As an example, consider a dog undergoing a major maxillectomy. With such a surgery, it is possible that thirst and hunger may result from interference with the dog's ability to eat and drink. However, the dog could be hand-fed and gradually taught to eat and drink despite the facial alteration. If the dog was never able to eat or drink again on its own, the owners could hand feed him for the rest of his life, or the dog could be fed via a feeding tube. While this may represent a compromise to his freedom to express normal eating behavior, he would not be hungry or thirsty. Such a surgery may result in a cure for the disease and eliminate pain. However, the surgery may also result in pain and distress. Fear and distress would result due to the unfamiliar surroundings and people during the hospital stay. With appropriate nursing care, the dog should not suffer any physical or thermal discomfort. This example illustrates how many factors are interrelated, and all need consideration in the cancer patient.

Of interest is the finding that the provision of analgesics significantly reduces the tumor-promoting effects of undergoing and recovering from surgery.[22] Surgery is well known to result in the suppression of several immune functions, including natural killer (NK) cell activity,[23,24] probably as a result of released cytokines, hormones, and inflammatory substances. This suppression of NK cell activity can enhance metastasis.[25] The reduction of the tumor-promoting effects of surgery by analgesics may result from maintenance of NK cell function, but it is likely that other unrecognized factors also play a role.[22] Thus, the provision of adequate perioperative pain management in oncological surgery may be protective against metastatic sequelae in clinical patients.

ASSESSMENT OF CANCER PAIN

Assessment of pain in animals, while often difficult, is extremely important. It is likely that the tolerance of pain by an individual animal varies greatly and is further complicated by the innate ability of dogs and cats to mask significant disease and pain. Physiological variables such as heart rate, respiratory rate, temperature, and pupil size are not reliable measures of acute perioperative pain in dogs[26] and are unlikely to be useful in chronic pain states. The mainstay of pain assessment in cats and dogs suffering from cancer is likely to be changes in behavior, and Table 16-2 outlines behaviors than are probably indicative, in certain situations, of pain. In general, if a tumor is considered to be painful in humans, it is appropriate to give the animal with a similar condition the benefit of doubt and treat it for pain.

One of the most useful ways of determining if a tumor is painful is to palpate the area and evaluate the animal's response. This may not correlate precisely with the amount of pain the animal spontaneously experiences, but if a tumor is painful on manipulation or palpation, it is highly likely there is spontaneous pain associated with it.

Veterinarians should involve technicians and other staff members in the assessment process as they are usually the ones spending the most time with the patients in the hospital and may have more time to converse in a relaxed and informal way with owners.

The most important people in the assessment process are the owners. The veterinarian must work closely with

TABLE 16-2	Outline of Behaviors that May Be Seen with Cancer-Associated or Therapy-Associated Pain in Cats and Dogs
Behavior	**Notes**
Activity level	Less activity, change in specific activities (e.g., decreased jumping, decreased play, less venturing outside and less willing to go on walks or lameness can all be associated with generalized pain).
Appetite	Often decreased with chronic pain.
Attitude	Any change in behavior can be associated with cancer pain—aggressiveness, dullness, shyness, "clinginess," increased dependence.
Facial expression	Head hung low, squinted eyes in cats; "sad" expression in dogs, head carried low.
Grooming	Failure to groom can be due to either a painful oral lesion or generalized pain.
Response to palpation	This is one of the best ways to diagnose and monitor pain. Pain can be elicited by palpation of the affected area, or manipulation of the affected area, which exacerbates the pain present. This is manifested as an aversion response from the animal (the animal attempts to escape the procedure, or yowls, cries, hisses, or bites).
Respiration	May be elevated with severe cancer pain.
Self traumatization	Licking or scratching at an area can indicate pain.
Urinary and bowel elimination	Failure to use litter box (cats); urinating and defecating in inappropriate areas (dogs).
Vocalization	Vocalization is rare in response to chronic pain in dogs and cats: however, owners of dogs will often report frequent odd noises (whining, grunting) associated with cancer pain. Occasionally cats will hiss, utter spontaneous plaintive meows, or purr in association with cancer pain.

the owner to capture this information. Often owners need to be educated as to what signs to look for and that certain behaviors may be indicative of pain. Once specific changes in behavior can be identified, and recorded, these can be used to monitor the effectiveness of analgesic therapy. This approach has proved sensitive in the evaluation of chronic pain due to osteoarthritis.[27] An example of the assessment sheet used at North Carolina State University for chronic osteoarthritis pain is shown in Table 16-3. The parameters that should be assessed have not been fully validated for osteoarthritis, although some progress in this respect has been made.[28-30] This has not been applied in cancer patients, but questions relating to interaction with family members, mood, responsiveness, dependence, response to handling, vocalization, ability to sleep, and restlessness, as well as activity-related questions, may all be appropriate. Importantly, the veterinarian needs to capture information about behaviors or activities that have changed as a result of the cancer. These are made as specific as possible to allow easy evaluation by the owner. At each assessment, these activities are evaluated and the option is given to evaluate others.

The author's approach to the evaluation of pain at each visit is to evaluate each of three aspects:
1. Palpation-induced pain
2. Activity parameters (using the client-specific outcome measures approach outlined above)

3. Behavioral parameters (using the client-specific outcome measures approach outlined above)

Follow-up is important, and the assessment of activity and behavioral parameters can be evaluated over the telephone.

PRINCIPLES OF ALLEVIATION OF CANCER PAIN

Pharmacologic Therapy

Drugs are the mainstay of cancer pain management, although nondrug adjunctive therapies are becoming recognized as increasingly important. The World Health Organization has outlined a general approach to the management of cancer pain[31] based on the use of the following "groups" of analgesics:
- Nonopioid analgesics (e.g., nonsteroidal antiinflammatory drugs, acetaminophen)
- Weak opioid drugs (e.g., codeine)
- Strong opioid drugs (e.g., morphine)
- Adjuvant drugs (e.g., corticosteroids, tricyclic antidepressants, anticonvulsants, N-methyl D-aspartate (NMDA) antagonists)

The general approach of the World Health Organization ladder is a three-step hierarchy (Figure 16-1).

TABLE 16-3	**An Example of the Assessment Sheet Used at North Carolina State University for Chronic Osteoarthritis Pain**				
Client specific outcome measures – activity Indicate how problematic these activities are compared to when your cat was normal, or did not have osteoarthritis. Comparison is to when he/she was 6 years old.					
Problems in Mobility Related to Osteoarthritis in Your Cat	**No Problems**	**A Little Problematic**	**Quite Problematic**	**Severely Problematic**	**Impossible**
1. jumping on green sofa		✓	✗		
2. jumping on kitchen counter			✓		✗
3. walking up steps on back deck	✓		✗		
4. jumping on bed	✓				✗
5. using litter tray	✓	✗			

Specific activities or behavior altered by the disease are defined, and the degrees of impairment or abnormality are determined at the first visit (✗).
The same activities or parameters are assessed after analgesic treatment is started (✓). A shift to the left corresponds to pain relief.
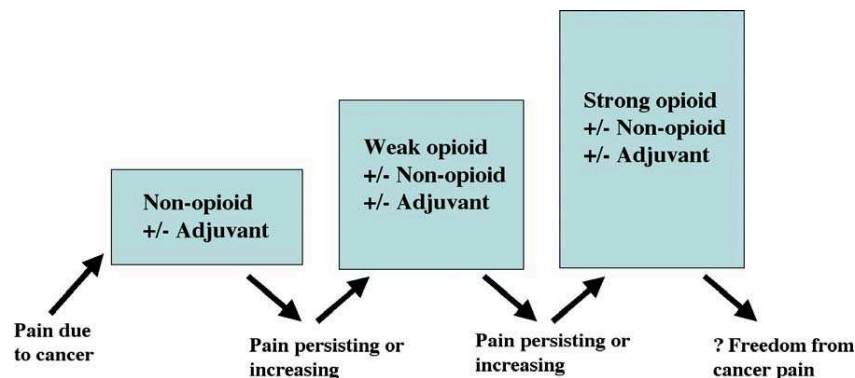
Figure 16-1

The World Health Organization's (WHO) "analgesic ladder" for the treatment of cancer pain. The three-step ladder is based on the premise that doctors and health care professionals should learn how to use a few drugs well. The three standard analgesics making up this ladder are NSAIDs, weak opioids (such as codeine), and strong opioids (such as morphine). Alternatives are substituted as necessary. This simple approach is likely to be superseded by a more complex approach based on the mechanism of pain in that particular cancer. The three-step WHO ladder approach is not always suitable for veterinary patients who often present with relatively advanced disease and significant pain.

Within the same category of drugs, there can be different side effects for individuals. Therefore, it may be best to substitute drugs within a category before switching therapies. It is always best to try to keep dosage scheduling as simple as possible. The more complicated the regimen, the more likely that noncompliance will occur. Drugs should be dosed on a regular basis, not just as needed, as pain becomes moderate to severe. Continuous analgesia will facilitate maintaining patient comfort. Additional doses of analgesics can then be administered for breakthrough pain. Adjuvant drugs can be administered to help with specific types of pain and anxiety.

There are two potential problems with the use of the WHO analgesic ladder in veterinary medicine. First, there is little information from human medicine, and virtually none from veterinary medicine, on which drugs

are most effective for particular types of cancer pain. It may well be that third-tier drugs might be most effective for a particular condition and therefore used upfront.

The second problem is that the approach is not well suited to patients that present initially with significant to severe pain. Many veterinary cancer patients present at an advanced stage of disease and thus are already in moderate to severe pain. Once pain has been present for a period of time, changes take place in the central nervous system that alter the way pain signals are processed. This alteration in processing (central sensitization) makes analgesics less effective and requires that multiple classes of analgesics be used concurrently to minimize pain. This is known as *multimodal pain therapy*. Once the pain is minimized and central changes are partially reversed, the amounts and types of drugs being administered may be decreased. This approach has been termed the *analgesic reverse pyramid approach*.[32] It is currently unknown which of these two approaches (the WHO ladder or the reverse pyramid) is most appropriate and, indeed, one approach may be best at one disease stage and the other later on.

The most important aspect to remember in the treatment of cancer pain is that for the majority of situations, *multimodal therapy* (i.e., concurrent use of more than one class of drug) *is required for the successful alleviation of the pain*.

A general outline to approaching cancer pain is given in Figure 16-2. This figure can be used in combination with Tables 16-4 and 16-5. The clinician may face many different scenarios when dealing with cancer pain, and these figures and tables are only a guide. The term *wind-down therapy* refers to admitting refractory pain patients into the hospital and treating them with multiple intravenous analgesic medications in the hope that the pain can be controlled, making it easier to subsequently manage the pain at home.

DRUGS USED FOR MANAGEMENT OF PAIN IN CANCER PATIENTS

The drugs that can be used for chronic cancer pain management are outlined in Tables 16-4 and 16-5. The following notes are not a comprehensive appraisal of each class of drug but are suggestions for their use for cancer pain.

Nonsteroidal Antiinflammatory Drugs (NSAIDs)

The cyclo-oxygenase enzyme (COX) exists in (at least) two different forms. One COX enzyme (COX-1) produces *essential prostaglandins* (e.g., prostaglandins that are involved in maintaining mucosal integrity of the stomach) on a minute-to-minute basis, and another (COX-2) is activated by tissue trauma and results in the production of *inflammatory* or *pain-mediating prostaglandins*. However, it is not as simple as COX-1 being *good* and COX-2 *bad*, as COX-2 has been inconsistently shown to be expressed constitutively in certain tissues such as the canine kidney,[33,34] canine kidney cells,[35] and the canine central nervous system (Lascelles BDX, unpublished data). It is not fully understood what role, if any, COX-2 plays in canine gastrointestinal mucosa. Simplistic theory would suggest that selective or preferential COX-2 inhibitors (e.g., carprofen, deracoxib, meloxicam, and firocoxib) might be associated with fewer gastrointestinal side effects than a nonselective drug, although widespread clinical experience is required before that can be ascertained. Theory would also been the mainstay suggest that COX-2 selective drugs are *not* any safer to the kidney than are the nonselective drugs.[36] Very often, cancer patients are at greater risk of toxic side effects from NSAIDs due to preexisting disease or concurrent therapy.

More recently, research has shown COX-2 plays a significant role in the development and progression of cancer. The production of prostaglandin E2 by COX-2 has been linked to the promotion of tumorigenesis.[37] COX-2 overexpression in cells will inhibit apoptosis, allowing neoplastic cells to continue to live. Other mechanisms by which COX-2 contributes to tumor development include the facilitation of adhesion and increasing the invasiveness of cancer cells, increased cell growth, and suppression of the immune system, and enhanced angiogenesis.[38]

COX-2 overexpression has been demonstrated in a number of premalignant and malignant conditions including colon cancer, non-small-cell lung cancer, breast cancer, bladder cancer, pancreatic cancer, prostate, and head and neck cancer in people.[39-45] Overexpression of COX-2 in tumors is being linked with a poor prognosis and more aggressive cancer. As a result of these findings, selective COX-2 inhibitors are being used in chemotherapy protocols against these cancers.[46,47] NSAIDs are also being used to protect against cancer in people genetically at risks for certain cancers, such as colon cancer.[48,49] Although it seems that some NSAIDs may be more effective than others.[50] Basic research has also shown that COX-2 inhibitors may enhance the effects of radiation of tumors.[51]

Significant research has recently been directed toward examining the role of COX-2 in dogs with cancer. In dogs, COX-2 is overexpressed in a number of carcinomas including bladder[52] and renal,[52] mammary,[53] intestinal,[54] cutaneous (squamous cell carcinoma)[55] nasal, and in some sarcomas such as osteosarcoma.

Recently, a number of investigators have looked at the anticancer properties of NSAIDs (to date, mainly only piroxicam has been examined) in canine tumors.
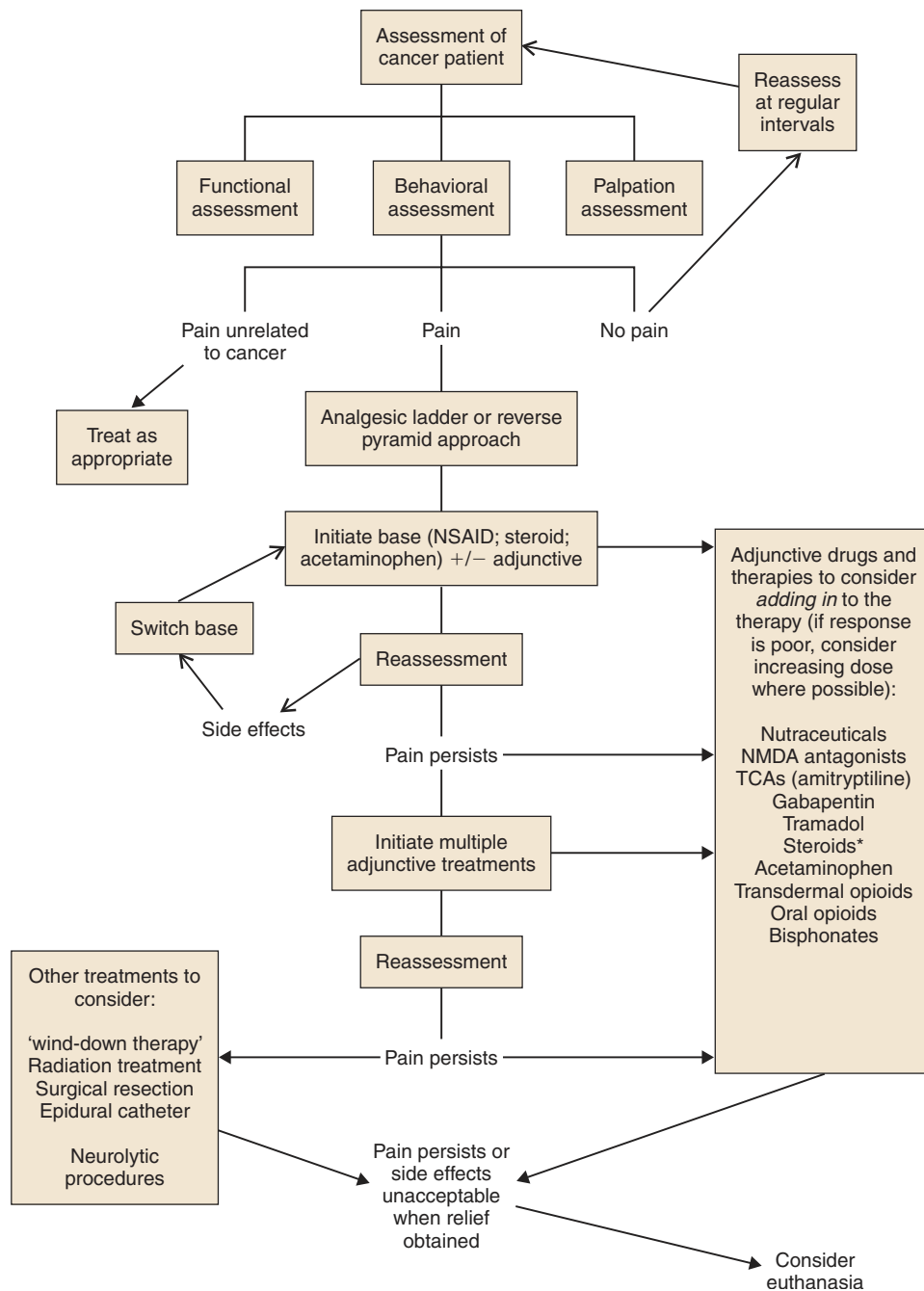
Figure 16-2

A decision tree giving a general outline to treating cancer pain in dogs and cats.
*Not combined with NSAIDs.

Piroxicam has been shown to cause regression of and slow the progression of rectal tubulopapillary polyps [56] Piroxicam has also been shown to have anticancer effects in canine transitional cell carcinoma, [57-61] and also in canine oral squamous cell carcinoma. [62] In multi-centric lymphoma, there was no apparent benefit to adding piroxicam to doxorubicin. [63]

There is now widespread interest in defining the anticancer effects of the available NSAIDs in canine and feline tumors, particularly now that there are approved selective or specific inhibitors of COX-2 for use in dogs (carprofen, deracoxib, firocoxib, meloxicam). Studies evaluating these drugs are ongoing in a number of centers.

TABLE 16-4   Analgesics for the Alleviation of Chronic Cancer Pain in the Dog

None of the drugs have been specifically evaluated for efficacy in the treatment of cancer pain. None of these drugs are approved or licensed for use in chronic cancer pain. Some drugs are approved for use in osteoarthritis, and doses are extrapolated from recommended doses for the treatment of osteoarthritis. (The doses given come from the author's experience, and the experience of others working in the area of clinical cancer pain control.)

Drug	Dose for Dogs	Comments
Acetaminophen	10-15 mg/kg PO q 8 hr for 5 days	Associated with less gastrointestinal side effects than regular NSAIDs and not noted to be associated with renal toxicity.
	Long-term therapy: up to 10mg/kg every 12 hr	Toxicity has, however, not been evaluated clinically in dogs. Can be combined with regular NSAIDs in severe cancer pain, but this combination has not been evaluated for toxicity
Acetaminophen (300 mg) + codeine (30 or 60 mg)	Dose on 10-15 mg/kg of acetaminophen.	Sedation can be seen as a side effect with doses at or above 2 mg/kg of codeine.
Amantadine	4.0-5.0 mg/kg PO q 24 hr	An NMDA antagonist and seems to produce significant level of analgesia when given in combination with a NSAID
		Loose stools and excess GI gas can be seen at higher doses. Should not be combined with selegiline or sertraline until more is known about drug interactions. Should not be used in seizure patients or in patients with heart failure.
Amitryptiline	0.5-2.0 mg/kg PO q 24 hr	Augments the descending serotinergic system. It is not a "strong" analgesic, but, like amantadine, tramadol, and codeine, is useful when combined with a NSAID or paracetamol. It has not been evaluated for clinical toxicity in the dog. Should not be used with tramadol.
Aspirin	10 mg/kg PO q 12 hr	Significantly more gastrointestinal ulceration than approved NSAIDs. *Caution: Avoid perisurgical use due to inhibition of platelet function.*
Butorphanol	0.2-0.5 mg/kg PO up to q 8 hr	May produce sedation at higher doses. Unpredictable analgesic, especially in the dog, and best used in combination with other analgesics (e.g., NSAIDs).
Codeine	0.5-2.0 mg/kg PO q 8-12 hr	Sedation can be seen at the higher doses.
Carprofen	2 mg/kg PO q 12 hr or 4 mg/kg PO q 24 hr	Some work suggests it is a preferential COX-2 inhibitor.
Deracoxib	1-2 mg/kg PO q 24 hr	As a specific COX-2 inhibitor, laboratory evidence suggests it should not be used in animals with existing gastrointestinal ulcers as it may inhibit healing.
Etodolac	5-15 mg/kg PO q 24 hr	
Fentanyl, transdermal	2-5 mcg/kg/hr	Can be useful in the short-term control of cancer pain. For long-term therapy, usefulness is limited due need to change the patch every 3 to 5 days.
Firocoxib	5 mg/kg q 24 hr	As a specific COX-2 inhibitor, laboratory evidence suggests it should not be used in animals with existing gastrointestinal ulcers as it may inhibit healing.

TABLE 16-4 Analgesics for the Alleviation of Chronic Cancer Pain in the Dog—cont'd

Drug	Dose for Dogs	Comments
Gabapentin	3-10 mg/kg PO q 6-12 hr	Has not been thoroughly evaluated in dogs as an analgesic. Is rapidly metabolized in the dog. Can be useful for certain neuropathic pain, such as after limb amputation, or nerve root tumor pain and other chronic pain/hypersensitivity conditions. Best effects are seen when used in combination with other analgesics such as NSAIDs or paracetamol (acetaminophen).
Glucosamine and chondroitin sulfate	13-15 mg/kg chondroitin sulfate PO q 24 hr	Can be used in a variety of cancer pains due to its mild antiinflammatory and analgesic effects. Best used in combination with a NSAID or other analgesic.
Meloxicam	0.2 mg/kg PO day 1, then 0.1 mg/kg q 24 hr	Considered a slightly preferential COX-2 inhibitor
Morphine, liquid	0.2-0.5 mg/kg PO q 6-8 hr	Can be useful for dosing smaller dogs where tablets are not suitable. Sedation and constipation are side effects that are seen as the dose is increased.
Morphine, sustained release	0.5-3.0 mg/kg PO q 8-12 hr	Doses higher than 0.5-1.0 mg/kg are often associated with unacceptable constipation, so suggest using 0.5 mg/kg several times a day. Certain preparations may be poorly absorbed or rapidly metabolized in dogs and may not provide analgesia.
Pamidronate (one of the bisphosphonate class of drugs)	1-1.5 mg/kg over 2 hr I.V. with ≈200 cc normal saline q 4-6 weeks	This drug inhibits osteoclast activity and thus likely provides analgesia in from primary or metastatic bone tumor with osteolysis.
Piroxicam	0.3 mg/kg PO q 24-48 hr	Some advocate the use of q 48 dosing to decrease the likelihood of GI toxicity.
Prednisolone	0.25-1 mg/kg PO q 12-24 hr; taper to q 48 hr if possible after 14 days	Do *not* use concurrently with NSAIDs. Can be useful in providing analgesia when there is a significant inflammatory component associated with the tumor and for CNS or nerve tumors.
Tepoxalin	10-20 mg/kg PO on day 1, followed by 10 mg/kg daily	This NSAID is a "dual inhibitor" that inhibits both cyclooxygenase and lipoxygenase enzymes. It is likely to be useful in cancer pain where there is a significant inflammatory component.
Tramadol	4-5 mg/kg PO q 6-12 hr	May be useful adjunctive analgesic (when combined with other analgesics such as acetaminophen or the NSAIDs) for the treatment of cancer pain in dogs.

NSAIDs have been the mainstay of therapy for chronic pain, especially in osteoarthritis. The choice of NSAIDs available can be bewildering, but here are a few key points to consider:

- On a population basis, all NSAIDs are equally efficacious in relieving pain associated with osteoarthritis, but for a given patient, one drug is often more effective than another.
- Gastrointestinal side effects associated with NSAID use appear to be more common with drugs that preferentially block COX-1 over COX-2.
- There is no difference in renal toxicity between COX-1 selective drugs and COX-2 selective drugs.
- Liver toxicity can occur with any NSAID.

- There are no completely safe NSAIDs, but the approved NSAIDs are significantly safer than the older nonapproved NSAIDs

The patient on NSAIDs should be monitored for toxicity by informing the owner of potential toxicity and what signs to watch for (lethargy, depression, vomiting, melena, increased water ingestion) as well as through the regular evaluation of blood work (and urinalysis) to evaluate renal function and liver function. A baseline should be obtained when therapy is initiated and parameters monitored on a regular basis thereafter. The author repeats evaluations after 2 to 4 weeks and then at 1 to 4 monthly intervals as dictated by the individual patient and client.

TABLE 16-5	Analgesics for the Alleviation of Chronic Cancer Pain in the Cat		

None of the drugs have been evaluated for efficacy in the treatment of cancer pain. None of these drugs are approved or licensed for use in chronic cancer pain. (The doses given come from the authors' experience, and the experience of others working in the area of clinical cancer pain control. Where sources of information are not given, there is no information on this drug as an analgesic in the cat.)

Drug	Cat Dose (mg/kg)	Notes	Published Source of Information/Reference on Analgesic Efficacy in Cats
Acetaminophen	Contraindicated	Contraindicated—small doses rapidly cause death in cats.	—
Amantadine	3.0-5.0 mg/kg PO q 24 hr	This drug has not been evaluated for toxicity in cats. May be a useful addition to NSAIDs in the treatment of chronic cancer pain conditions. The 100 mg capsules need to be recompounded for cats.	[a]
Amitriptyline	0.5-2.0 mg/kg PO q 24 hr	Well tolerated for up to 12 months of daily administration. Occasionally drowsiness is seen (<10%). May be a useful addition to NSAIDs for treatment of chronic pain conditions.	94,95
Aspirin	10 mg/kg PO q 48 hr	Can cause significant gastrointestinal ulceration.	—
Buprenorphine	0.02 mg/kg sublingual q 6-7 hr	May produce anorexia after 2-3 days and smaller doses (5-10 mcg/kg) may be more appropriate for "long-term" administration.	75
Butorphanol	0.2-1.0 mg/kg PO q 6 hr	Generally considered to be a poor analgesic in cats except for visceral pain; however, the author has found it to be useful as part of a multimodal approach to cancer pain therapy.	106
Carprofen	Not enough data to enable recommendations for long-term administration	—	—
Etodolac	Not recommended	—	—
Flunixin meglumine	1 mg/kg PO single dose		
Glucosamine/ Chondroitin sulphate combinations	Approx 15 mg/kg chondroitin sulphate PO q 12 to 24 hr	This combination appears to produce mild antiinflammatory and analgesic effects in cats. Can be used in conjunction with NSAIDs, opioids, and amantadine.	
Ketoprofen[b]	1 mg/kg PO q 24 hr; maximum 5 days	Probably well tolerated as pulse therapy for chronic pain, with a few days "rest" between treatments. Has been used by some at 1 mg/kg every 3 days long term.	[a]

TABLE 16-5   Analgesics for the Alleviation of Chronic Cancer Pain in the Cat—cont'd

Drug	Cat Dose (mg/kg)	Notes	Published Source of Information/Reference on Analgesic Efficacy in Cats
Meloxicam	0.1 mg/kg PO on day 1, followed by 0.05 mg/kg PO daily for 4 days, then 0.05 mg/kg every other day or 0.025 mg/kg daily	This drug is particularly well received by cats due to its formulation as a honey syrup. The suggested dosing regimen has not been evaluated for clinical toxicity.	107
Morphine (oral liquid)	0.2-0.5 mg/kg PO t.i.d.-q.i.d.	Best compounded into a palatable flavored syrup; however, cats strongly resent this medication. Morphine may not be as effective in cats as it is in dogs.	
Morphine (oral sustained release)	Tablets too large for dosing cats	—	—
Piroxicam	1 mg/cat PO daily for a maximum of 7 days/ if longer term medication is considered, suggest every other day dosing, but see note at right	This can be compounded into a palatable liquid; however, recent information suggests that the active drug decreases significantly over a 10-day period after compounding into an aqueous solution. In the authors' experience, significant drops in PCV (presumably due to GI hemorrhage) occur in up to 30% of cats after 2-3 weeks of drug therapy.	[a]
Prednisolone	0.25-0.5 mg/kg PO q 24 hr	Can be particularly effective in cancers associated with significant inflammation (such as squamous cell carcinoma of the oral cavity in cats). ***Not* to be combined with concurrent NSAID administration**	[a]
Tolfenamic acid[b]	4 mg/kg PO q 24 hr for 3 days maximum	—	—
Tramadol	1-2 mg/kg twice daily	Not yet extensively used for the treatment of cancer pain in cats.	[a]
Transdermal fentanyl patch	2-5 μg/kg/hr	A 25 μg/hr patch can be applied to an "average" cat (7.7-11 lb; 3.5-5.0 kg). In smaller cats, other methods of providing analgesia should be sought as it is not recommended to cut patches in half. The decay in plasma levels following patch removal is slow.	108, 109
Vedaprofen	Not recommended	—	—

Adapted from Lascelles, B. D. X., Drug therapy for acute and chronic pain in the cat. Int J Pharm Compounding 6:338-343, 2002.

[a]From authors' and colleagues' experience.

[b]Drug indicated is licensed and approved for use at the stated dose in one of the following countries: United States, United Kingdom, Australia, New Zealand.

NSAIDs in cats

Cats have generally longer, but variable and inconsistent rates of metabolism and excretion of NSAIDs compared to other species. The majority of the kinetic studies performed in cats have been carried out on single doses,[64-67] and no studies have yet examined the metabolism of chronically administered NSAIDs. Given the fact that most of the NSAIDs have a relatively long half-life in cats, chronic dosing is likely to be more dangerous for cats than for dogs.

There are no licensed NSAIDs for chronic administration (>5 days) in cats, although a number can probably be used safely (see Table 16-5). The key to safe chronic NSAID administration in cats is the use of the smallest effective dose and avoiding use, or using decreased doses, in cats with renal disease.

Additions to the use of NSAIDs for cancer pain relief

If pain relief with NSAIDs is inadequate, oral opioid medications, such as morphine or tramadol, can be administered. Acetaminophen or acetaminophen/codeine combinations can often be used in conjunction with NSAIDs. Transdermal fentanyl can also be used. Fentanyl, morphine, or tramadol can be used for dogs that cannot be given NSAIDs. Other agents that are used to treat chronic pain include amantadine (an NMDA antagonist); anticonvulsants, such as gabapentin; and tricyclic antidepressants, such as amitriptyline. These can all be combined with NSAIDs.

Acetaminophen

Acetaminophen (paracetamol) is a non-acidic NSAID; many authorities do not consider it an NSAID as it probably acts by different mechanisms. Although its mechanism of action is poorly understood, it has recently been suggested that it acts on a new variant of the cyclo-oxygenase enzyme COX-1 V1,[68] which is present in central nervous system tissues.[69,70] With any chronic pain, there are always central nervous system changes, so for what seems a "peripheral" problem, such as many cancers, centrally acting analgesics can be very effective. Although highly toxic in the cat, even in small quantities, it can be effectively used in dogs for pain control. No studies of toxicity in dogs have been done, but if toxicity is seen, it will probably affect the liver, so the drug should be used cautiously in dogs with liver dysfunction. It can be used on its own or in a preparation combined with codeine and is initially dosed at about 10 to 15 mg/kg bid. The authors often use it as the first line of analgesic therapy in dogs with renal compromise where NSAIDs cannot be used or in dogs that appear to be otherwise intolerant to NSAIDs (e.g., vomiting or gastrointestinal ulceration).

Opioids

Opioids can be an effective part of the management of cancer pain, particularly when used as part of a multimodal approach (i.e., including NSAIDs or adjunctive analgesics). Side effects of opioids include diarrhea, vomiting, sedation, and constipation with long-term use. It is often constipation, and occasionally the sedation, that owners object to most as a side effect in their pet. Oral morphine, transdermal fentanyl, oral butorphanol, sublingual buprenorphine (cats only) and oral codeine are used most often for the alleviation of chronic cancer pain. None of these drugs has been fully evaluated for clinical toxicity when administered long term or for efficacy against chronic cancer pain. Dosing must be done on an individual basis, and adjustment of the dose to produce effective analgesia without undesirable side effects requires interaction and communication with clients. Evidence from North Carolina State University has indicated that certain preparations of prolonged release oral morphine and oral methodone may not reach effective plasma concentrations in dogs when dosed at the currently recommended levels.[71,72]

Opioids in cats

There is currently no information on the long-term use of oral opioids for chronic pain in the cat. There appears to be individual variation in the level of analgesia obtained with certain opioids, especially morphine and butorphanol, in the acute setting.[73,74] Buprenorphine appears to produce predictable analgesia when given sublingually[75] and is well accepted by most cats. The small volume required (max 0.066 ml/kg [20 micrograms/kg]) makes administration simple. Based on clinical feedback from owners, this is an acceptable technique for home use. Inappetance can occur after several days of treatment, and sometimes lower doses (5 to 10 micrograms/kg) can overcome this problem. When administered concurrently with other drugs, less frequent dosing is often required.[75]

NMDA Antagonists

The N-methyl D-aspartate (NMDA) receptor appears to be central to the induction and maintenance of central sensitization,[76-78] and the use of NMDA receptor antagonists would appear to offer benefit where central sensitization has become established (i.e., especially chronic pain).[79] Ketamine, tiletamine, dextromethorphan, and amantadine possess NMDA antagonist properties, among other actions.

Injectable ketamine is not obviously useful for the management of chronic pain due to the need for frequent injections and the tendency for dysphoric side effects even at low doses. However, oral ketamine has not been evaluated in dogs or cats for long-term administration.

Intraoperative "microdose" intravenous ketamine appears to provide beneficial effects for a variety of oncology surgical procedures, including limb amputations,[80] and this may decrease the incidence of chronic pain later on. Other reports suggest a benefit of using ketamine perioperatively in low doses.[81] When used in this manner, ketamine should be administered as a bolus (0.5 mg/kg IV) followed by an infusion (10ug/kg/min) prior to and during surgical stimulation. A lower infusion rate (2 ug/kg/min) may be beneficial for the first 24 hours postoperatively and an even lower rate (1 ug/kg/min) for the next 24 hours. In the absence of an infusion pump, ketamine can be mixed in a bag of crystalloid solutions for administration during anesthesia. Using anesthesia fluid administration rates of 10 ml/kg/hr, 60 mg (0.6 ml) of ketamine should be added to a 1-liter bag of crystalloid fluids to deliver ketamine at 10 ug/kg/min.

The active metabolite of dextromethorphan may not be produced in dogs, probably negating its use in the species for chronic pain.[82]

Amantadine has been used for the treatment of neuropathic pain in humans.[83,84] The author has been using amantadine over the past few years as an adjunctive drug for alleviating chronic pain, and objective evaluation of its clinical use is currently under way. It is used as an adjunct to NSAIDs and appears to augment pain relief with a low incidence of side effects (mainly agitation and diarrhea over the first few days of administration). It often takes 7 to 14 days to have a positive effect and may be used long term. Suggested doses are given in Tables 16-4 and 16-5. The toxic side effects have been evaluated in the dog (but not the cat), and the doses suggested are considered safe.[85] Amantadine should be avoided in patients with congestive heart failure, those with a history of seizure, or those on selegiline, sertraline, or tricyclic antidepressants.

Combination Analgesics

Tramadol is a synthetic derivative of codeine and is classified as an opioidergic/monoaminergic drug.[86,87] It has been found to be effective in the alleviation of pain associated with osteoarthritis in humans.[88-91]

Tramadol has action at the mu opioid receptor and also facilitates the descending serotonergic system. Tramadol has been used for perioperative pain in animals and is occasionally used for chronic pain. The doses given in Tables 16-4 and 16-5 are for the regular (not prolonged release) form of the drug. It has not been thoroughly evaluated for toxicity in the dog or cat.

Anticonvulsant Drugs

Gabapentin is a structural analogue of GABA (gamma-aminobutyric acid) and was introduced as an antiepileptic drug. It appears to provide analgesia by modulating the alpha 2 delta subunits of calcium channels, thereby decreasing calcium influx. It appears to be useful for neuropathic pain and central sensitization in some patients and has recently been demonstrated to be analgesic in relevant models of bone cancer pain.[92,93] It is metabolized rapidly in the dog and is most often used for its anticonvulsive properties. It appears to have some analgesic properties at low doses administered two or three times daily.

Tricyclic Antidepressants

The tricyclic antidepressant amitriptyline appears to be effective in the cat for pain from interstitial cystitis,[94,95] and many practitioners are reporting efficacy in other chronically painful conditions in the cat and dog. Amitriptyline has been used daily for periods up to 1 year, and few side effects were reported. It should not be used concurrently with tramadol until more is known about drug interactions.

Steroids

Steroids have a mild analgesic action, can produce a state of euphoria, and are often used for these reasons to palliate cancer and cancer pain in cats and dogs. They should not be used concurrently with NSAIDs because the risk of side effects (especially gastrointestinal) is increased dramatically.

Bisphosphonates

Bone pain induced by primary or metastatic bone tumors is thought to be due primarily to osteoclast activity. Drugs, like the bisphosphonates, that block osteoclast activity can markedly reduce bone pain.[96,97] There is little information on their use in dogs for palliation of bone pain, but drugs such as pamidronate and zoledronate are starting to be used, and early information suggests a decrease in pain in about 40% of cases.[2]

Other Pain-Relieving Modalities

Local or whole-body radiation can enhance analgesic drug effectiveness by reducing metastatic or primary tumor bulk.[98] Intravenous administration of strontium-89 has also been shown to provide analgesia related to bony metastases in approximately 50% of humans, but its use is uncommon in veterinary patients.

Acupuncture can be used as a pain-relieving modality in conjunction with conventional therapy. It is often useful with other therapy to allow lower doses of drugs that may have significant side effects.[99-101] Neutraceuticals contain a variety of compounds, but the main ones used are preparations with glucosamine and chondroitin sulphate. These are often used in osteoarthritis,

and there is evidence that they provide a mild anti-inflammatory effect and an analgesic effect. A more complete discussion of the use of acupuncture and neutroceuticals can be found in Chapter 17.

THE FUTURE: TOWARD A MECHANISTIC UNDERSTANDING OF CANCER PAIN

Over the past few years, it has become evident that the pain transmission system is plastic—that is, it alters in response to inputs. This plasticity results in a unique neurobiological signature within the peripheral and central nervous system for each painful disease. Understanding the individual neurobiological signatures for different disease processes should allow novel, targeted, and more effective treatments to be established.[102] This approach should also allow for a more informed choice to be made regarding which of the currently available drugs might be most effective.

The *first relevant model* of cancer pain has been established in rats—an osteosarcoma model.[103] Prior to this model, evaluation of mechanisms and treatments were undertaken in chronic pain models such as sciatic nerve ligation or injection of chronic irritants—models that did not involve cancer. New clinically relevant models have allowed targeted pain treatments to be developed, such as the use of osteoprotegerin for bone cancer pain.[96] Such models will also allow for the screening of other novel treatments such as neuroablation techniques, or substance P–saporin combination.[104,105]

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SECTION B
Nutritional Management of the Cancer Patient

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Optimal nutrition plays an important role in the successful management of cats and dogs with neoplastic disease. However, differences in the biologic behavior of various tumors as well as wide individual variation in preexisting nutritional status and nutrient requirements means that no single diet is appropriate for every cat or dog with cancer. This section of the chapter examines the complex interaction between nutritional status and the tumor-bearing state. Practical methods for nutritional assessment of the individual animal will be discussed, followed by a description of the various methods available to provide appropriate nutrition. The primary purpose of this section is to provide a logical and practical algorithm by which the nutritional needs of the individual cat or dog with malignant disease can be defined and met.

CANCER AND NUTRITION

The complex relationship between nutrition and cancer has two distinct components. The negative effect of malignant disease on nutritional status is well described in many species, and the clinical recognition and treatment of this problem in cats and dogs with cancer is the main focus of this chapter. However, it is increasingly clear that nutrition, diet, and nutritional status play a primary or contributing role in the pathogenesis of many types of cancer. The precise pathways by which nutrient excess or deficiency modulate malignant transformation are poorly understood, but cancer prevention through nutrition is an extremely active and important area of research. A detailed discussion of this subject is beyond the scope of this article, but what is known about the specific role of nutrition in tumor pathogenesis in cats and dogs is briefly discussed at the end of the chapter.

Cancer Cachexia

The negative impact of cancer on host nutritional status is so well established and accepted that a specific term, *cancer cachexia*, is usually used to describe the weight loss associated with malignant disease. Cancer cachexia is most simply defined as severe weight loss in an individual with neoplasia (Figure 16-3).[1] However, this basic definition overlooks the highly variable clinical

Figure 16-3

A dog with severe cancer-associated weight loss.

presentation of cancer cachexia, as well as its multifactorial etiology. Cancer cachexia is characterized clinically by involuntary weight loss, fatigue, anemia, and progressive depletion of both lean body mass and adipose stores.[2] Even though weight loss must obviously be present for a diagnosis of cancer cachexia to be considered, affected people and animals will not necessarily exhibit all of these typical clinical signs. Further, specific clinical signs can vary significantly in severity from individual to individual. Finally, clinical signs related to cancer cachexia are also superimposed on clinical signs specific to the patient's histopathologic diagnosis and stage of disease. The obvious result is that no two animals with cancer cachexia are exactly the same, and if the veterinarian fails to preserve some index of suspicion for the syndrome, affected cats and dogs will be missed.

Cancer cachexia is common in people, although its prevalence varies with tumor type.[1] In one study of more than 3000 patients, the frequency of weight loss in the 6 months prior to a diagnosis of cancer varied from a low of 32% in people with treatment-responsive non-Hodgkin's lymphoma to a high of 87% in people with measurable gastric tumors.[3] Cancer cachexia was most commonly associated with cancers of the upper gastrointestinal tract, such as gastric and pancreatic carcinomas.

Weight loss is clinically significant in the cancer patient because of its detrimental effect on quality of life and prognosis.[1,4-6] The weakness associated with cancer cachexia means that affected people may be unable to complete even simple tasks needed for daily living.[2,6] Affected individuals are less able to tolerate aggressive therapy for their cancers, at least in part because compromised nutritional status can significantly impact the pharmacokinetics and pharmacodynamics of chemotherapeutics and contribute to increased treatment-related toxicity.[1,6] Regardless of tumor type, people with cancer

cachexia are also less likely to respond to treatment for their underlying malignancy and have significantly shorter survival times compared to weight-stable individuals.[3,6,7] As a form of protein-energy malnutrition, cancer cachexia causes hypoproteinemia, delayed wound healing, and immunosuppression, and it leads to significant compromise of gastrointestinal, pulmonary, and cardiovascular function.[1,4] Death is the eventual and inevitable result, unless the underlying tumor can be successfully eradicated. Malnutrition is arguably one of the most common causes of death in people with cancer.[5,6,8,9]

Pathogenesis of cancer cachexia

Cancer-associated weight loss can be divided into two major categories: primary and secondary.[5,6] The underlying causes of secondary cancer cachexia are functional in nature and common to many neoplastic and non-neoplastic diseases. It is intuitive how most secondary causes of cachexia result in weight loss. For instance, the anatomic location of some primary or metastatic tumors clearly has a direct and negative impact on food intake, ability to digest, or capacity to absorb nutrients. Two feline tumors, oral squamous cell carcinoma and gastrointestinal lymphoma, are good examples of malignancies that can lead to this type of weight loss. It is also obvious that nutrient intake, digestion, and absorption are significantly compromised in some patients by the side effects of antineoplastic therapy. Early satiety develops in approximately 50% of people receiving therapy for cancer.[8] Chemotherapy can also decrease food intake by causing alterations in taste and smell perception and may lead to the development of learned food aversions.[10] While similar phenomena have not been specifically documented in cats and dogs with cancer, they are likely to occur. People and animals receiving chemotherapy may also experience nausea, vomiting, and anorexia because of drug stimulation of neuroreceptors in the gastrointestinal tract or the central nervous system. Both chemotherapy and radiotherapy have the ability to cause injury to the rapidly proliferating epithelial cell populations that line the gastrointestinal tract as well. The result can be stomatitis or gastroenteritis severe enough to lead to decreased food intake, dehydration, maldigestion, and malabsorption.[1,5,6]

Primary cancer cachexia, on the other hand, is a poorly understood paraneoplastic syndrome that has been described in people and animals with malignant disease. Appropriate assisted feeding for whatever deficiencies are present should lead to improved nutritional status in animals and people with secondary cancer cachexia. However, primary cancer cachexia cannot be completely reversed through increased nutrient intake or assisted feeding, and this distinguishes it from other types of weight loss that occur with neoplastic disease.[11,12] Affected individuals may experience weight loss in the face of an apparently adequate voluntary food intake. The underlying pathophysiology of primary cancer cachexia is complex and incompletely defined. Tumor-related changes in host metabolism appear to lead to inefficient use of energy, which leads to lean body mass and adipose stores being depleted. The intermediary metabolism of any or all of the three energy substrates—fat, protein, and carbohydrate—may be abnormal. The result is that no matter how many calories are fed or by what route they are provided, the patient's requirements cannot be met.[5]

Neoplastic disease could result in primary cancer cachexia by two basic mechanisms. It is possible that substances synthesized and released by the tumor itself interfere with host intermediary metabolism.[1] Primary cancer cachexia may also be the result of an abnormal or exaggerated neural, endocrine, or cytokine response to the presence of neoplastic disease in a tumor-bearing host that prevents normal use of consumed calories and eventually leads to weight loss.[13] For instance, neuroendocrine abnormalities such as altered plasma and urine catecholamine and cortisol concentrations, as well as changes in both the relative and absolute concentrations of plasma insulin and glucagon, have been demonstrated in rodents with implanted tumors and people with cancer cachexia.[1,6,14-16] More recent studies, however, suggest that primary cancer cachexia may be an inflammatory disorder caused by alterations in a large and variable group of proinflammatory mediators that have wide-ranging effects on intermediary metabolism. Interleukin-1α (IL-1α), IL-1β, IL-6, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and the eicosanoids have all been implicated in the pathogenesis of the syndrome.[1,5,6,9,13,16-18] Changes in the production of these substances could be responsible in turn for the observed changes in the hormonal milieu.[15] Regardless, attempts to specifically identify the underlying cause of primary cancer cachexia have been complicated by the fact that multiple factors contribute to weight loss, and this combination of factors is likely unique to each individual and varies over the course of the disease.

The biochemical abnormalities seen in weight-losing rodents with implanted tumors and people with naturally occurring primary cancer cachexia have classically reflected abnormalities in carbohydrate metabolism. These include hyperlactatemia, increased rates of whole-body glucose turnover and disposal, increased rates of gluconeogenesis from lactate and amino acids, abnormal glucose tolerance curves, hyperinsulinism, and insulin resistance.[1,5,6,9,14,19] Documented changes in protein metabolism have consisted of altered serum amino acid profiles, increased rates of whole-body protein turnover, decreased protein fractional synthetic rates in skeletal muscle, and increased protein fractional synthetic rates in liver.[1,5,6,9,13,14,16] Finally, abnormalities

in lipid metabolism have included accelerated fat oxidation, increased lipolysis, hypertriglyceridemia, decreased lipoprotein lipase activity, increased synthesis of triacylglycerols and very low density lipoproteins (VLDL), and increased plasma concentrations of nonesterified or free fatty acids and ketone bodies (acetoacetate and beta-hydroxybutyrate).[1,5-7,13,14,16,20]

While there is little published information regarding the metabolic impact of the tumor-bearing state in the cat, many of the biochemical or metabolic abnormalities considered typical of primary cancer cachexia in people have been demonstrated in dogs with various neoplastic diseases. Interestingly, these abnormalities often do not resolve after the primary tumor has been treated. Significantly higher serum lactate and insulin concentrations have been documented in dogs with lymphoma as compared to normal controls, although glucose tolerance curves were not different between these two groups.[21] In another study, hyperlactatemia and hyperinsulinemia did not resolve when dogs with lymphoma achieved a complete clinical remission after doxorubicin chemotherapy.[22] Dogs with a variety of nonhematopoietic tumors have also been shown to have increased serum lactate and insulin concentrations after an intravenous glucose tolerance test. Once again, these abnormalities did not resolve after apparently complete surgical resection of disease in the tumor-bearing dogs.[23] Altered lipoprotein profiles were found in a group of dogs with untreated lymphoma, including increased very low density lipoprotein (VLDL) cholesterol, total triglyceride, VLDL triglyceride, low density lipoprotein (LDL) triglyceride, and high density lipoprotein (HDL) triglyceride. HDL cholesterol was decreased in these dogs. Several of these changes persisted even when dogs achieved complete clinical remission after chemotherapy, and the authors speculated that alteration in lipoprotein lipase activity may have been the cause.[24] Finally, dogs with osteosarcoma were demonstrated to have increased urinary nitrogen excretion and whole-body glucose flux in the period immediately after amputation, although their whole-body protein synthetic rates were decreased.[25]

Regardless of species, it has been hypothesized that the fundamental reason for weight loss in primary cancer cachexia is increased energy expenditure. It should be apparent from the biochemical abnormalities discussed earlier that energy metabolism in the tumor-bearing host is frequently characterized by futile cycling and increased flux through many metabolic pathways, any of which should lead to an increase in metabolic rate and energy expenditure.[9] Accordingly, the energy expenditure of tumor-bearing dogs, rodents, and people has been measured by many investigators using indirect calorimetry, in an attempt to prove that hypermetabolism is responsible for cancer-associated weight loss. Indirect calorimetry is a technique in which oxygen consumption and carbon dioxide production are measured and used to calculate the subject's respiratory quotient and energy expenditure. Unfortunately, indirect calorimetry studies do not provide consistent support for the presence of an increased metabolic rate in subjects with malignant disease; the results obtained have varied widely and must be interpreted carefully. For example, in one study 15 weight-stable large breed dogs with naturally occurring osteosarcoma were demonstrated by indirect calorimetry to have relatively increased resting energy expenditure both before and after amputation when compared to 12 healthy, 1-year-old intact female laboratory beagles.[25] In contrast, the same group of investigators could find no differences in resting energy expenditure among dogs with various solid tumors including osteosarcoma, either when resting energy expenditure was compared before and after tumor excision or when the resting energy expenditure of the tumor-bearing dogs was compared to that of client-owned control dogs.[26] Finally, a third study showed that dogs with lymphoma actually had decreased resting energy expenditure when compared to normal control dogs, whether they were in clinical remission or not.[27] There are several plausible explanations for this kind of variability in indirect calorimetry data and apparent resting energy expenditure. First, the control groups chosen for these studies by various investigators are not uniform: weight-losing cancer patients are compared to weight-stable cancer patients in some studies, while others use weight-losing subjects with nonmalignant disease or even completely normal individuals as controls. Second, it is reasonable to expect that metabolic rate and thus energy expenditure vary with tumor type, stage of disease, and preexisting nutritional status.[1] Finally, the extremely sensitive equipment used to perform indirect calorimetry yields results that can be difficult to reproduce consistently. Considerable expertise is necessary to determine when the subject is in the steady state needed to obtain accurate measurements, and three to five measurements are recommended over a 24-hour period in order to obtain a complete assessment.[28] The end result is that while the predicted increase in energy expenditure can be documented among weight-losing cancer patients in some studies, energy expenditure is normal in others and actually decreased in still others.[1,6,13,14] More studies are needed to fully define how hypermetabolism plays a role in cancer-associated weight loss.

Prevalence and clinical significance of cancer cachexia in cats and dogs

Most veterinary studies have failed to show a convincing association between documented metabolic abnormalities, actual weight loss, and poor prognosis in cats or dogs with neoplastic disease. As discussed in detail earlier, weight loss is common in people with cancer, and when it is caused by primary cancer cachexia, it should be

accompanied by one or more of the previously described biochemical abnormalities. Based in part on demonstration of some of these characteristic biochemical changes in dogs with malignant disease, it has been assumed that weight loss is equally common in veterinary patients. However, a study examining dogs evaluated and treated at a referral oncology practice suggests this may not be the case.[29] Only 4% of the dogs in this study were cachectic as defined by body condition scoring, while 29% were obese; 15% of the dogs had detectable and clinically significant muscle wasting. Documented weight loss was present in 68% of dogs, but in 31% of these dogs, it represented less than 5% of the animal's body weight prior to the diagnosis. The authors concluded that weight loss appeared to be less common and less severe in dogs with neoplastic disease than in people. There are several potential explanations for these findings. First, it is possible that the types of cancer most often diagnosed or treated in dogs are different than those diagnosed and treated in people, and they are simply less likely to result in cancer cachexia. For instance, in this study the most frequent diagnosis was lymphoma, one of the tumor types least associated with weight loss in people. It is also probable that in veterinary practice, dogs having a favorable prognosis are more likely to receive treatment, whereas in people, therapy is generally attempted even in the face of a poor prognosis. This practice would tend to exclude some dogs that are likely to respond poorly to therapy and are therefore at greatest risk for weight loss. Finally, a major difference between cancer treatment in animals and in people is that cats and dogs with neoplastic disease are routinely euthanized when their quality of life becomes irreversibly unacceptable. It is impossible to gauge the specific effect of euthanasia on studies of this type, but it seems probable that some dogs are euthanized before they become cachectic. Regardless, it will be essential for future studies to specifically correlate the presence of clinically significant biochemical abnormalities considered typical of cancer cachexia with documented weight loss and poor outcome in cats and dogs with cancer. If tumor-bearing cats and dogs have the biochemical changes traditionally associated with cancer cachexia yet do not consistently lose weight, investigation into the reasons behind this lack of weight loss may prove a rewarding area for further research.

Nutritional assessment

Nutritional assessment is the clinical method used to diagnose all forms of malnutrition in cats and dogs with neoplastic disease, including cancer cachexia. Systematic application of this technique allows the veterinarian to accomplish four important tasks: it identifies and grades the severity of existing malnutrition; it predicts the risk of future malnutrition; it identifies individuals needing nutritional support; and it is used to evaluate the response to assisted feeding.[28]

Three primary areas are evaluated during the course of nutritional assessment in most cats and dogs. A diet history incorporating a detailed description of both the current and habitual diet (if these differ) is generally obtained first. Product brand names, ration form (i.e., canned, dry, or semimoist), and flavors, plus the amount fed and the frequency of feeding, should be included. Owners should be asked specifically about medications and nutritional supplements they are currently using, as well as any that have been given to the animal in the past. The duration and course of the underlying neoplastic disease and its potential impact on the animal's diet should also be considered. It is not unusual for owners to make significant changes in feeding practices after a diagnosis of cancer has been made in their pet, with or without veterinary advice. Typically, the goal of these changes is to maximize overall food intake and to take advantage of the perceived benefits provided by increased intake of a specific nutrient or nutrients. Regardless, a thorough evaluation of present nutritional status in any animal requires that precise information about changes in food intake over time be included in the diet history.

The second major component of nutritional assessment is a physical examination. All body systems should be carefully evaluated: as the physical abnormalities caused by malnutrition can be subtle and difficult to detect. The clinician must be aware that two major forms of malnutrition occur in cats and dogs with neoplastic disease: malnutrition caused by undernutrition (i.e., protein-energy malnutrition and cancer cachexia) and malnutrition caused by overnutrition (i.e., obesity). As already discussed, obesity is common among dogs with malignant disease, and a large subset of malnourished animals with cancer will be overlooked if the clinician is focused solely on identifying animals with weight loss.

The diagnosis of obesity is straightforward, but the physical abnormalities associated with cancer cachexia or protein-energy malnutrition can be highly variable and nonspecific. Physical examination of a cat or dog with cancer that also has inadequate intake of protein and calories may or may not reveal weight loss, muscle wasting, pallor, evidence of chronic infections, poor hair coat, and abnormal nail growth.[30,31] Peripheral edema is rare, but it can be present in advanced cases. Serial measurement of body weight is useful in documenting weight loss in affected animals; clearly, single body weight measurements will be less helpful in this regard.

Another limitation of body weight is that it does not provide information regarding body composition. Body composition is defined by the distribution of body mass between three separate compartments: fat-free tissue or lean body mass, extracellular water, and adipose tissue. Accurate measurement of the size of

these compartments yields a precise assessment of nutritional status and permits even mild or subclinical malnutrition to be diagnosed. Techniques used to determine body composition in people include standardized anthropometric measurements such as midarm muscle circumference and triceps skinfold thickness, radioisotope dilution methodology, determination of total-body potassium, dual x-ray absorptiometry (or DEXA) scanning, and hydrodensitometry.[5,28] These methods are generally not available for application in animals, although DEXA scanning is used in some research settings. Use of a standardized body condition scoring system provides a practical alternative and can supply the veterinarian with at least some of the same information about lean body mass and adipose stores in cats and dogs. Studies have confirmed that there is good correlation between body condition scoring and actual body composition as measured by DEXA scanning in canine and feline patients.[32-35] Body condition scoring systems utilize a 5- or 9-point system, where each point corresponds to a particular body condition as defined by specific criteria (Figure 16-4). Body condition scoring should reflect expansion of fat mass in obese cats and dogs with neoplastic disease, while cats and dogs with primary cancer cachexia should have depletion of both lean body mass and fat mass. However, this technique may not be sensitive enough to detect small changes in either compartment.[29]

The third and final step in nutritional assessment involves the evaluation of routine hematological and biochemical parameters, although it is important to note that none of these parameters by themselves are very sensitive or specific as a tool for the diagnosis of malnutrition. For instance, normocytic, normochromic, nonregenerative anemia is considered characteristic of cancer cachexia in people[36] and could be present in affected cats and dogs, although it also commonly occurs with many other disorders.[30] The serum biochemical abnormalities observed in the malnourished cat or dog with neoplastic disease vary dependent on whether the animal suffers from overnutrition or undernutrition. Hyperglycemia, glucose intolerance, hyperlipidemia, and increased serum concentrations of liver enzymes may be seen in obese cats and dogs.[37] Conversely, hypokalemia is common in cats that are anorexic from any cause.[30,38] Severe cancer cachexia with profound muscle wasting could also lead to increased serum creatine kinase concentrations in the cat.[39] Inadequate intake of protein could lead to decreased blood urea nitrogen and serum creatinine concentrations and may affect the serum biochemical parameters used to assess liver function.[30,40] Dietary protein deficiency has also been implicated as a factor in the pathogenesis of idiopathic feline hepatic lipidosis. The most characteristic finding among cats with hepatic lipidosis is a significant elevation in serum

alkaline phosphatase, but increased concentrations of hepatocellular transaminases, total bilirubin, and bile acids are often observed as well.[41-43]

Cats and dogs suffering from severe protein malnutrition associated with cancer cachexia could have decreased serum albumin and total protein concentrations.[30,40] Hypoproteinemia is a good indicator of protein malnutrition, and serum albumin concentration is the single most reliable nutritional predictor of a poor clinical outcome in critically ill people.[28] Since albumin has a relatively long serum half-life (approximately 8 days in the dog),[44] in the absence of dramatic concurrent losses a prolonged period of deprivation is necessary before serum albumin concentrations become subnormal. Short half-life serum proteins such as transferrin, fibrinogen, prealbumin, retinol-binding protein, and insulin-like growth factor-I are now used in people because they provide a more accurate and dynamic reflection of protein status than serum albumin alone.[28,45-47] The potential role of short half-life proteins in the nutritional assessment of cats and dogs with cancer cachexia has not yet been defined.

Feeding Cats and Dogs with Cancer

Whether self-supportive and in the home environment or critically ill and hospitalized, the target food intake for the cat or dog with cancer is almost always based on an estimation of the animal's energy requirements. This is an effective approach because virtually all commercial cat and dog foods are complete and balanced rations, formulated to meet needs for all essential nutrients except water when the animal consumes a quantity of the ration sufficient to meet its caloric requirement. Although careful attention must always be paid to fluid balance, additional supplements addressing specific nutrient deficiencies are indicated only under rare circumstances.

The five steps outlined here can be used to systematically define nutritional requirements and formulate an appropriate feeding protocol for the cat or dog with neoplastic disease.

1. Estimate fluid requirement

Water is essential for many metabolic processes and chemical reactions within the body and is also needed for thermoregulation. Normal cats and dogs require 50 to 100 mL of water per kilogram body weight per day, although this amount varies substantially with activity level, environment, and diet. For instance, water intake is less in cats and dogs that eat canned rations compared to dry foods because of the high moisture content of these products.[48] Providing free access to clean water at all times allows self-supportive cats and dogs with cancer to tailor their water intake to meet their requirements, but this approach is inadequate for

BODY CONDITION SCORING SYSTEM

1. VERY THIN

The ribs are easily palpable with no fat cover. The tailbase* has a prominent raised bony structure with no tissue between the skin and bone. The bone prominences are easily felt with no overlying fat. In animals over six months, there is a severe abdominal tuck when viewed from the side and an accentuated hourglass shape when viewed from above.

2. UNDERWEIGHT

The ribs are easily palpable with minimal fat cover. The tailbase* has a raised bony structure with little tissue between the skin and bone. The bony prominences are easily felt with minimal overlying fat. In animals over six months, there is an abdominal tuck when viewed from the side and a marked hourglass shape when viewed from above.

3. IDEAL

The ribs are palpable with a slight fat cover. The tailbase* has a smooth contour or some thickening and the bony structures are palpable under a thin layer of fat between the skin and the bone. The bony prominences are easily felt with a slight amount of overlying fat. In animals over six months, there is an abdominal tuck when viewed from the side and a well proportioned lumbar waist when viewed from above.

4. OVERWEIGHT

The ribs are difficult to feel with moderate fat cover. The tailbase* has some thickening with moderate amounts of tissue between the skin and bone. The bony structures can still be felt. The bony prominences are covered by a moderate layer of fat. In animals over six months, there is little or no abdominal tuck or waist when viewed from above. Abdominal fat apron present in cats.

5. OBESE

The ribs are very difficult to feel under a thick fat cover. The tailbase* appears thickened and is difficult to feel under a prominent layer of fat. The bony prominences are covered by a moderate to thick layer of fat. In animals over six months, there is a pendulous ventral bulge and no waist when viewed from the side. The back is markedly broadened when viewed from above. Marked abdominal fat apron present in cats.

*Tailbase evaluation is done only in dogs.

Figure 16-4

Five-point body condition scoring system for cats and dogs. *(Adapted from McCurnin, D. M., Clinical Textbook for Veterinary Technicians. Philadelphia, W.B. Saunders, 1998, p. 666.)*

many hospitalized animals. The fluid requirements of a critically ill cat or dog with neoplastic disease should be specifically calculated and compared against actual water intake to ensure that the animal's needs are being met. The calculated requirement should incorporate increased insensible fluid losses caused by conditions such as fever, hyperventilation, or hypermetabolism; excessive fluid losses associated with gastrointestinal or renal dysfunction; excessive loss from pleural or peritoneal effusions and wound drainage; and any preexisting fluid deficits. Either enteral or parenteral fluid supplementation may be appropriate, depending on the circumstances.

2. Estimate energy requirement

The amount of energy expended by healthy cats and dogs under various circumstances can be described in several different ways. The basal energy requirement (BER) or basal metabolic rate (BMR) is the amount of energy required by a resting but conscious, postabsorptive animal in a thermoneutral environment; it is sufficient to maintain essential cellular, cardiac, and respiratory functions. The resting energy requirement (RER) includes all components of the BER, plus an increment that supports some minor muscular activity such as standing up or lying down. Finally, the maintenance energy requirement (MER) consists of the RER plus the energy required to move, digest, and absorb food within the intestinal tract.[49]

Numerous equations have been developed to estimate MER in healthy cats and dogs, but there is controversy among nutritionists regarding which equations are most accurate. Several of these equations were originally developed using data collected in caged animals under laboratory conditions, and they may not be directly applicable to pet animals living in a home environment. It is also unclear that these equations remain appropriate after the superimposition of neoplastic disease on normal maintenance, because of the many complex issues that could affect energy expenditure in the tumor-bearing animal; additional study is needed in this area. Regardless, maintenance equations are probably a reasonable way to establish initial food intake for a self-supportive and weight-stable cat or dog with cancer. The value obtained for MER is simply used as a rough estimate of individual energy requirements, and ongoing assessment of body condition score is then used to adjust food intake as needed. MER is increased if the animal is losing weight and decreased if the animal is gaining weight. This repeated reassessment of response is essential to optimize food intake and maintain an ideal body condition score (see Figure 16-4). Although more complex exponential equations exist, the linear equations listed in Table 16-6 are practical and easy to use. They all estimate MER in kilocalories of metabolizable energy (ME) per kilogram body weight per day.[50,51]

TABLE 16-6	**Maintenance Energy Equations for Use in Adult Cats and Dogs**
Animal	**MER Equation**
Neutered adult dog	$[70 + 30(BW_{kg})] \times 1.6$
Intact adult dog	$[70 + 30(BW_{kg})] \times 1.8$
Obese-prone adult dog	$[70 + 30(BW_{kg})] \times 1.2$ to 1.4
Neutered adult cat	$[70 + 30(BW_{kg})] \times 1.2$ to 1.4
Intact adult cat	$[70 + 30(BW_{kg})] \times 1.4$ to 1.6
Obese-prone adult cat	$[70 + 30(BW_{kg})] \times 1.0$

The energy requirements of hospitalized animals with cancer are incompletely defined. Most authors use RER to estimate the energy expended by an anorexic, critically ill cat or dog confined to a hospital cage regardless of the underlying disease, although this approach obviously does not take into account the potential impact of the tumor-bearing state on energy requirements. As with equations for MER, RER equations only provide an initial estimate of the energy requirement for an individual animal: ongoing assessment of nutritional status is once again essential, and food intake should be adjusted as needed to maintain an ideal body condition score. The RER equations used to estimate initial energy requirements for hospitalized dogs and cats with neoplastic disease are as follows:[30]

$$RER \text{ (kcal metabolizable energy/day)} = 70 + 30(BW_{kg})$$
$$\text{(for animals from 2 to 45 kg)}$$

$$RER \text{ (kcal metabolizable energy /day)} = 70(BW_{kg})^{0.75}$$
$$\text{(for animals of any weight)}$$

The exponential equation is most accurate and should be used for very small and very large animals.

Some authors also multiply the calculated RER for hospitalized animals by an "illness factor." The purpose of this adjustment is to individualize energy intake by taking into account the severity of the animal's illness: energy needs are believed to be increased in people and animals with more critical illnesses.[52-54] However, if illness factors are used, it is safest to apply them conservatively. The actual energy requirement of a cat or dog with chronic anorexia and weight loss may be substantially less than expected due to adaptations that decrease metabolic rate to conserve energy.[6,30, 55-57] Furthermore, overly aggressive nutritional support has been associated with serious metabolic complications including cholestasis and hepatic lipidosis in both cats and people.[58-60] It has also been reported to cause hypermetabolism and

respiratory failure in people.[61,62] The author uses illness factors ranging from 1.0 to 1.2 times the RER in most cases; illness factors up to a maximum of 1.4 times RER in the cat and 1.5 to 1.6 times RER in the dog are occasionally used for disease states such as disseminated malignancy with concurrent sepsis. Representative illness factors are listed in Table 16-7.

Finally, the results of nutritional assessment should always be considered when establishing initial energy requirements for individual cats and dogs with neoplastic disease. Animals with documented weight loss, low body condition scores, and supporting evidence for protein-energy malnutrition on clinical pathology will generally require caloric intakes greater than the calculated RER or MER to regain optimal nutritional status. Repeated nutritional assessment with incremental increases in caloric intake as needed based on the animal's documented response is the best way to avoid overfeeding and its associated complications. On the other hand, a conservative reduction in calculated caloric intake may be indicated for obese cats and dogs in order to gradually achieve an ideal body condition score and optimal nutritional status. The deleterious clinical effects of obesity in small animals are well established and include musculoskeletal disease, glucose intolerance and diabetes mellitus, immunosuppression, and shortened life span.[37,63] Regardless of the benefits of weight loss, an aggressive weight reduction protocol should not be instituted during critical illness, even in extremely obese animals. Severe caloric restriction could contribute to the rapid development of clinically significant protein-calorie malnutrition or "stressed starvation,"[55] with loss of lean body mass and associated complications such as hypoproteinemia,

delayed wound healing, immunosuppression, and compromised organ function. A better approach is to initiate a conservative weight loss program once the animal's condition has stabilized. The specific steps involved in formulating a successful weight loss program for an obese cat or dog have been previously described.[37]

3. Distribute calories

A huge variety of prescription and over-the-counter pet foods that may be appropriate for a cat or dog with cancer are available, and a practical way to narrow the choices for an individual animal is to first select a target range for distribution of the calories to be provided between protein, fat, and carbohydrate. The optimal caloric distribution is determined by a number of factors including the results of nutritional assessment, the specific underlying histopathologic diagnosis of cancer, and the existence of concurrent diseases. These factors all vary from animal to animal, which in turn means that no single ration will meet the needs of every cat or dog with cancer. Instead, each animal must be carefully evaluated based on objective criteria so that a diet recommendation that best fits the specific situation can be made. Commercial rations recommended for weight-losing cats and dogs with neoplastic disease and otherwise normal organ function generally supply 30% of calories as protein. A high-fat intake is often recommended as well, for several reasons. High-fat diets theoretically take advantage of the metabolic differences between tumor and normal host cells by preferentially supplying energy to host cells, since neoplastic cells are thought not to possess the metabolic machinery required to oxidize fats. Rations high in fat have been shown to normalize carbohydrate metabolism and modestly prolong survival times in some dogs with lymphoma.[64] Another benefit of a high-fat diet is that after more than 24 hours of anorexia, the biochemical response to food deprivation in cats and dogs leads to substantial dependence on fat-derived fuels. Feeding a ration high in fat simply takes advantage of existing metabolic adaptations.[30] Fat also provides more calories per gram (8.5 kcal/gm) than protein (3.5 kcal/gm) or carbohydrate (3.5 kcal/gm).[65] The increased energy density of a high-fat ration is advantageous in sick animals with marginal voluntary food intakes as well as animals being supported with feeding tubes because more calories are delivered in a smaller volume. Finally, rations high in fat tend to be more palatable, which may improve voluntary food intake in animals with inconsistent appetites. Most cats and dogs can tolerate up to 65% of their total energy requirement as fat, as long as there is a period of adaptation.[30]

The commercial rations that are most likely to fit this general profile (30% protein calories, up to 60% fat

TABLE 16-7	Illness Factors for Use with the Calculated Resting Energy Requirement (RER) in Critically Ill and Hospitalized Cats and Dogs
Condition	**Adjustment to RER**
Hospitalization	1.0
Elective surgery	1.0 to 1.2
Trauma	1.0 to 1.3
Neoplastic disease	1.0 to 1.3
Sepsis	1.0 to 1.4
Major thermal burns	1.2 to 1.6[a]

[a]Maximum illness factor 1.4 times the RER in cats.

Adapted from Slatter, D., Textbook of Small Animal Surgery. Philadelphia, W.B. Saunders, 2003, p. 92.

calories, with the remainder as carbohydrate) are prescription products formulated for use during performance or stress, "premium" cat and dog foods, and puppy or kitten foods. Prescription rations formulated for use in critically ill cats and dogs are listed in Table 16-8. A prescription product marketed specifically for dogs with neoplastic disease (n/d, Hills Pet Nutrition Inc., Topeka, Kansas) is also available; this product is high in fat and protein content, and it is enriched with n-3 fatty acids.

It is important to recognize, however, that a high-protein, high-fat diet is not appropriate for every cat or dog with cancer. This type of ration is contraindicated in animals with a documented history of fat intolerance (i.e., vomiting, diarrhea, pancreatitis, or hyperlipidemia), regardless of the presence of underlying neoplastic disease. It will also make preexisting obesity more difficult to manage. Choice of ration will be further impacted by concurrent diseases that also require dietary management, for example, renal insufficiency: in this case a phosphorus and protein restricted ration would be preferred over a product with a relatively higher protein content. When protein or fat intake must be restricted, carbohydrate calories can be

TABLE 16-8	**Commercial Enteral Feeding Products for Use in Cats and Dogs**					
Product Name	Manufacturer	Protein (g/100 kcal ME)	Fat (g/100 kcal ME)	Carbohydrate (g/kcal ME)	Caloric Density	Special Features
Clinicare	Abbott Animal Health	8.2	5.1	6.8	1 kcal/mL	Added n-3 fatty acids, glutamine, arginine, and taurine; lactose free; pourable
Maximum-Calorie Canine Dry[a]	Eukanuba Veterinary Diets	7.8	5.6	4.4	634 kcal/cup	Added n-3 fatty acids and branch chain amino acids; dry formulation
Maximum-Calorie Canned Canine and Feline[b]	Eukanuba Veterinary Diets	7.5	7.1	1.3	2.1 kcal/mL	Added n-3 fatty acids, branch chain amino acids, and taurine; syringable
Canine/Feline a/d, Canned[b]	Hill's Pet Nutrition	9.0	6.2	3.5	1.3 kcal/mL	Added n-3 fatty acids, glutamine, branch chain amino acids, potassium, and taurine; syringable
Canine n/d, Canned[a]	Hill's Pet Nutrition	7.0	6.1	3.7	660 kcal/14.75 oz can	Added n-3 fatty acids and arginine
CV Feline Formula, Canned[b]	Nestlé Purina	8.8	5.5	4.7	223 kcal/5.5 oz can	Low sodium; added potassium, carnitine, and taurine

[a]*Formulated for use in dogs only.*

[b]*Formulated for use in both cats and dogs.*

Note: The information included is based on data supplied by the manufacturers.

substituted, although tumor-bearing animals may not use sugars efficiently because of insulin resistance and glucose intolerance. Overt diabetes mellitus is an obvious contraindication for high intake of simple sugars.

Once the optimal distribution of calories for an individual animal has been determined, the commercial rations fitting this profile are identified. A final selection is made after considering additional factors including digestibility, fiber content, palatability, necessity for tube feeding, cost, and convenience. Prior episodes of food intolerance as well as strong preferences by the animal with regard to diet formulation (dry, canned, or semi-moist) or flavor should also be taken into account.

4. Evaluate remaining nutrients

The best way to meet basic requirements for vitamins, minerals, and other essential nutrients such as taurine is to use a complete and balanced commercial ration specifically formulated for the species of animal eating the food. A food that has passed Association of American Feed Control Officials (AAFCO) feeding trial testing is preferred. Some cats and dogs with cancer may also have specific nutrient requirements that are known or suspected to be higher than normal. Individual nutrients that are often supplemented in small animals, whether or not they have neoplastic disease, include potassium, phosphorus, iron, vitamin K, the B vitamins, carnitine, and taurine.[31]

Some authors have also advised supplementing certain other nutrients that are proposed to provide unique benefits for small animals with neoplastic disease. The dietary constituents that have been studied in the greatest detail with respect to cats and particularly dogs with cancer are n-3 fatty acids, although the amino acids glutamine and arginine, as well as various antioxidants are frequently recommended as well. What is currently known about each of these nutrients is summarized next, but further work in this area is needed. Optimal dosing, dose scheduling, and duration of nutritional therapy are for the most part unknown. Many of the studies published to date describe the impact of feeding commercial prescription diets enriched with several different nutrients to dogs with neoplastic disease. Unfortunately, this approach results in multiple concurrent diet changes and makes it difficult to definitively attribute any observed benefits to a particular nutrient.

n-3 Fatty Acids. Most prescription pet foods intended for use in critically ill cats and dogs, including those with underlying cancer, are supplemented with n-3 fatty acids. Manipulation of the dietary ratio of n-3 to n-6 fatty acids alters the fatty acid composition of cell membranes throughout the body and has several potential benefits. The type of eicosanoid that is produced from cell membrane fatty acids profoundly affects the inflammatory cascade. The series 4 leukotrienes and the

series 2 prostaglandins are derived from the n-6 fatty acid arachidonic acid, and are pro-inflammatory. On the other hand, the series 5 leukotrienes and the series 3 prostaglandins that are synthesized from the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) competitively inhibit metabolites of arachidonic acid and are less potent stimulators of inflammation. Studies have shown that the inflammatory response in the dog can be altered by increasing dietary intake of n-3 fatty acids: lower dietary n-6 to n-3 fatty acid ratios decrease synthesis of the pro-inflammatory leukotriene B isomer LTB_4, and increase synthesis of the less inflammatory LTB_5 in canine neutrophils and skin.[66] In addition, attenuation of the inflammatory response has been reported in atopic dogs consuming an n-3 fatty acid enriched diet.[67,68]

Pro-inflammatory cytokines such as IL-1, TNF-α, and IL-6 are principal mediators of inflammation, and the mechanism by which n-3 fatty acids affect the inflammatory response is most likely through alteration of the synthesis of these and perhaps other cytokines.[69] Cytokine production was significantly decreased by n-3 fatty acid supplementation in both young and old women, possibly through n-3 fatty acid interaction with eicosanoid production.[70] Altered cytokine production resulting from consumption of an n-3 fatty acid enriched diet has been proposed as the mechanism underlying enhanced body condition in dogs with cardiac cachexia.[71] Supplementation with n-3 fatty acids has been shown in several studies to decrease production of pro-inflammatory cytokines and stabilize weight loss in people with pancreatic cancer,[1,72,73] and it ameliorates weight loss in cachectic tumor-bearing mice as well.[74,75] Finally, people with disseminated malignancy that received n-3 fatty acids in the form of fish oil capsules had restored immune function, decreased *in vitro* production of TNF by peripheral blood mononuclear cells, and prolonged survival compared to controls.[76]

Rations enriched with n-3 fatty acids may benefit cats, dogs, and people with cancer by mechanisms other than modulation of inflammation.[77,78] Favorable outcomes have been reported in dogs with lymphoma fed an experimental diet supplemented with n-3 fatty acids and arginine. Consumption of this diet corrected some abnormalities in carbohydrate metabolism, as demonstrated by decreased lactic acid production and insulin release after intravenous glucose and diet tolerance tests. Disease free intervals and survival times after chemotherapy among dogs with WHO stage IIIa lymphoma eating the test diet were improved as well, although dogs with WHO stage IVa lymphoma did not enjoy similar benefit.[64] The improved outcome among some of the dogs in this study is difficult to attribute to amelioration of cancer cachexia, the biochemical changes classically associated with cachexia, or even to n-3 fatty acid supplementation alone. These dogs did not have weight

loss, so they did not, by definition, have cancer cachexia. Furthermore, no significant differences in serum TNF-α, IL-6, or the acute phase reactant alpha-1 acid glycoprotein, were found between dogs eating the test diet and dogs eating a control diet, so blunting of an inflammatory response that might theoretically lead to cancer cachexia could not be documented. Finally, study subjects were supplemented with both n-3 fatty acids and arginine, so it is also impossible to separate the effects of these two nutrients. The authors hypothesized that the prolongation in disease free interval and survival time that they observed with stage IIIa lymphoma dogs was the result of increased intake of polyunsaturated fatty acids (PUFAs) in the dogs eating the test diet. PUFAs are reported to have direct antitumor activity, perhaps secondary to lipid peroxidation and production of superoxide radicals; they also inhibit tumor cell proliferation and promote apoptosis.[64]

It is worthwhile to note that despite potential advantages, risks may be associated with dietary supplementation of n-3 fatty acids in small animals. Increased intake of n-3 fatty acids leads to decreased synthesis of thromboxane A_2 and increased production of prostaglandin I_3 in people, which in turn causes significant decreases in platelet aggregation and vasoconstriction.[79] While this effect might decrease the incidence of atherosclerotic disease in people and might even inhibit the metastatic cascade, it could also predispose some animals to hemorrhage. So far, this does not appear to be a clinically relevant problem in the dog. While slight decreases in platelet aggregation have been reported in dogs consuming a diet moderately enriched with n-3 fatty acids,[80] in another study there was no alteration in platelet function among dogs with lymphoma or hemangiosarcoma that were fed an experimental diet supplemented with n-3 fatty acids.[81] However, larger declines in platelet function were reported in cats fed a ration containing a high level of n-3 fatty acids, and this species may be more likely to experience clinically significant changes in coagulation.[82] Diets containing large quantities of n-3 fatty acids may also depress normal immune function. Decreased lymphocyte proliferation has been documented in women given n-3 fatty acid supplements,[70] and significant suppression of cell-mediated immunity has been observed in dogs given n-3 fatty acid supplements.[83] This effect has been attributed to increased tissue lipid peroxidation caused by ingestion of large quantities of polyunsaturated fats in the form of n-3 fatty acids. However, vitamin E deficiency may also play a role since the amount of vitamin E required by subjects given n-3 supplements to protect against oxidative damage may be increased.[70,83] In addition, it has been suggested that wound healing is compromised by high intake of n-3 fatty acids, although the authors of at least one study were unable to find histologic evidence of compromised wound healing in dogs supplemented with n-3 fatty acids.[84] Additional work will be necessary to further define the most appropriate doses, ratios, and indications for n-3 fatty acids in cats and dogs with cancer.

Glutamine. Glutamine is the most abundant free amino acid in plasma and intracellular pools. It constitutes approximately 20% of total circulating free amino acids, and 60% of total intracellular free amino acids in skeletal muscle. Although glutamine is considered a nonessential amino acid, it plays a central role in several metabolic pathways. Glutamine contains two nitrogen groups and serves as a major means of nitrogen transport between tissues. It is a primary substrate for ammonia synthesis in the kidney and is also required for synthesis of nucleotides and numerous other macromolecules. Glutamine is an important energy substrate for the intestinal mucosa and is required by lymphocytes and other rapidly dividing cell populations.[85,86]

Marked decreases in plasma and free intracellular glutamine concentrations in skeletal muscle occur in critically ill people. Intracellular glutamine in skeletal muscle represents an essential storage pool of labile carbon and nitrogen that can be rapidly mobilized to meet the increased requirements of many tissues during protein-calorie malnutrition.[85-90] Intracellular glutamine may also play a role in the regulation of protein turnover. Depletion of intracellular glutamine is followed by a decrease in the rate of muscle protein synthesis and accelerated protein catabolism.[91,92] Several studies suggest that a high intake of glutamine during illness helps prevent loss of lean body mass by promoting and supporting muscle protein synthesis and decreasing muscle protein catabolism.[93-95] However, the role of glutamine in the maintenance of normal gut and immune system function may be even more important for the critically ill animal.[30,95-99] Glutamine is now considered by many investigators to be a conditionally essential nutrient during protein-calorie malnutrition, required in quantities that are greater than those that can be synthesized by the body.[14] Based on this hypothesis and preclinical studies performed in dogs,[100-103] the commercial veterinary critical care rations often recommended for cats and dogs with cancer are routinely supplemented with glutamine. Glutamine supplementation has also been suggested as a way to promote more rapid resolution of acute side effects of the oral mucosa in dogs receiving oronasal radiotherapy[104] and to maintain gut immunity and integrity in people receiving radiotherapy or chemotherapy.[95,105] Further study is needed to define the role of glutamine supplementation for cats and dogs with cancer.

Arginine. Arginine is an essential amino acid in cats and dogs. Although previously considered nonessential in people, studies now suggest that endogenous arginine

synthesis is inadequate to maintain nitrogen balance in some situations.[105-107] Arginine plays a critical role in urea and collagen synthesis; stimulates the release of hormones such as insulin, growth hormone, and insulin-like growth factor-1; modulates immune function; and promotes wound healing.[105] Supplementation has been shown to enhance collagen synthesis and improves wound breaking strength.[108-110] Arginine also has an important role in immune function. Lymphocytes have an absolute requirement for arginine, and supplementation increases lymphocyte mitogenic response, improves T cell function, and appears to enhance *in vitro* and *in vivo* antitumor cytotoxicity in rodents and people.[106,107,111-113] Arginine supplementation in critically ill people with and without cancer has resulted in decreased rates of postoperative wound infection and decreased duration of hospitalization.[107,112,114-116] While the specific benefits of arginine supplementation in cats and dogs with cancer are unknown, similar benefits are possible. Dogs with WHO stage IIIa lymphoma fed an experimental diet supplemented with both arginine and n-3 fatty acids had modest improvements in disease free intervals and survival times compared to dogs eating a control diet; however, there was no apparent effect in dogs with stages other than IIIa.[64] Several of the commercial rations intended for use in critically ill cats and dogs and often recommended for animals with neoplastic disease are enriched with arginine.

Folate, B$_{12}$, and Antioxidants. People that regularly consume large quantities of fruits and vegetables appear to have a significantly decreased risk of cancers such as carcinoma of the lung, head and neck, and upper gastrointestinal tract.[117-121] Several nutrients could be responsible for this protective effect, including folate, B$_{12}$ and the antioxidant vitamins A, C, and E. In addition to its role in the pathogenesis of cancers of the upper aerodigestive tracts, it is hypothesized that folate deficiency may be a factor in the development of carcinomas of the breast, colon, and cervix.[122-126] Significant improvement in cervical dysplasia has been documented after folate supplementation in women,[126] and increased intake of folate and B$_{12}$ reduces bronchial squamous metaplasia in smokers.[127] High-dose folic acid has also been shown to protect beagle dogs against chemically induced gastric carcinogenesis.[128] However, no published studies have yet examined the folate or B$_{12}$ status of cats and dogs with naturally occurring neoplastic disease.

Folate deficiency is believed to promote carcinogenesis through at least two potential mechanisms: DNA hypomethylation and uracil misincorporation. Folate in the form of 5-methyltetrahydrofolate normally participates in the synthesis of methionine by donating a methyl group to homocysteine. Methionine is then converted to S-adenosylmethionine (SAM), which is required for normal methylation of DNA. When folate is deficient these conversions are blocked, leading to hyperhomocysteinemia and DNA hypomethylation. DNA hypomethylation in turn causes altered gene expression[129] and may alter the expression of various oncogenes including c-myc, c-fos, and H-ras. Hypomethylation of these important regulatory genes is presumed to lead to uncontrolled cellular proliferation and subsequent tumorigenesis.[130] Uracil misincorporation is another mechanism by which folate deficiency may promote carcinogenesis. Folate in the form of 5,10-methylenetetrahydrofolate normally donates a methyl group to uracil, yielding the thymine needed for repair and synthesis of DNA. During folate deficiency uracil is incorporated into DNA in place of thymine, and this substitution may lead to permanent DNA breaks, chromosome damage, and ultimately mutagenesis.[122]

Antioxidants including vitamins A, C, and E, beta-carotene, and lutein are now added in increased quantities to many commercial pet foods, and anticancer claims are made for some of these products. Chronic oxidative stress with formation of reactive oxygen species, especially when antioxidant capacity is inadequate, has been hypothesized to contribute to DNA damage, malignant transformation, and eventual tumor development in numerous species. However, very little is known about the potential antineoplastic effect of antioxidant supplementation in pet cats and dogs. Preliminary work in dogs with mammary tumors revealed that the degree of lipid peroxidation was greater in tumor tissue compared to adjacent normal tissues, although increased antioxidant activity was found within the tumors assayed as well.[131] Dietary selenium supplementation also appeared to decrease DNA damage and promote apoptosis in non-neoplastic prostatic epithelial cells in geriatric laboratory beagles.[132] The potential role of antioxidants in tumor prevention has been studied much more extensively in people. Unfortunately, while low dietary intake of antioxidants has been associated with an increased risk of various types of cancer in people,[133] the results of chemoprevention studies have been disappointing. It is not clear that simple supplementation with antioxidants yields the same results as long-term consumption of the same vitamins and minerals in their naturally occurring forms in foodstuffs.[117] Furthermore, recent large studies in people have either found no decrease in cancer incidence,[134,135] or have documented significantly increased rates of primary tumor recurrence or second primary cancers during supplementation with beta-carotene or vitamin E.[136,137] More work remains to be done before antioxidant supplementation can be confidently recommended for tumor chemoprevention in a clinical setting.

5. Choose a method of feeding

Voluntary Intake. Voluntary intake is the preferred method for feeding cats and dogs with cancer. It is practical

and cost effective, and it is the least labor intensive of all available techniques. However, the veterinarian must take care not to overestimate actual intake in an anorexic or partially anorexic animal. When multiple meals and foods are offered concurrently to an inappetant cat or dog, the true quantity of food consumed may be difficult to assess. The only way to meet an animal's nutritional needs with certainty is to start by defining those needs in detail and follow by measuring the quantities of food and water consumed as accurately as possible. If there is a discrepancy between the volumes of food and water needed to meet the animal's requirements and the volumes taken in, some form of assisted feeding is indicated. Nutritional support should be initiated as early as possible in the clinical course of disease, since even a short period of food deprivation may lead to complications associated with protein-energy malnutrition.[6,138]

Pharmacologic Appetite Stimulation. Pharmacologic appetite stimulation has the potential to be a convenient and relatively cost-effective means of nutritional support. However, it is dangerous to simply assume that a prescribed appetite stimulant has increased food intake as expected: accurate measurement of the volume of food consumed is the only way to confirm drug efficacy. Clinically significant malnutrition may develop if an appetite stimulant is assumed to be working when it is not and an unacceptable delay in the institution of appropriate assisted feeding is possible if a prolonged period is spent waiting for an ineffective drug to provide benefit. There are very few controlled clinical studies in the veterinary literature that provide objective evidence of the efficacy of drugs such as diazepam or cyproheptadine in stimulating appetite in cats or dogs with cancer. Megestrol acetate is a more effective appetite stimulant in people and is commonly prescribed for the treatment of cancer-associated anorexia.[1,139-141] But there are no controlled trials describing the use of this drug in anorexic cats or dogs with neoplastic disease. Megestrol acetate has been associated with the development of diabetes mellitus[142] and mammary adenocarcinoma in the cat,[143,144] so it should be used with caution in this species. Pharmacologic appetite stimulants are most appropriately viewed as a last resort for cats and dogs with cancer, and when they are used the clinician must be committed to measuring food intake to confirm the desired effect. The efficacy of these drugs is unproven, and their use should be reserved for cases in which other means of assisted feeding for the truly anorexic animal are not an option. Techniques such as hand feeding of small frequent meals and the use of highly palatable and nutrient dense rations should also be attempted first.[11,145]

Assisted Enteral Feeding. Assisted feeding for the cat or dog with cancer can be provided enterally, parenterally, or with a combination of these two routes.

Enteral feeding is almost always preferred, because it allows nutrients to be metabolized through normal pathways.[6,138,146,147] In addition, food within the intestinal tract plays a critical role in maintaining gut health and function. Enteral nutrition supports normal biliary IgA secretion, preserves gut-associated lymphatic tissue (GALT), decreases bacterial translocation, prevents intestinal mucosal atrophy, maintains normal intestinal myoelectric activity, and improves intestinal healing and adaptation after bowel resection.[6,96,97,99,138,146,148-152] Enteral nutrition is easier to administer than parenteral nutrition, has fewer potential complications, and is generally less expensive. Finally, there is some disconcerting evidence that parenteral feeding may stimulate tumor growth and the development of metastatic disease.[138,153]

Indwelling tubes that are used to deliver enteral support in cats and dogs with cancer include nasoesophageal, pharyngostomy, esophagostomy, gastrostomy, and enterostomy tubes (Figure 16-5). The surgical techniques for placement of these tubes are described in detail elsewhere.[31] Enteral support should always be provided by the simplest route for which there is no contraindication, and food should be allowed to pass through as much of the length of the gut as possible. Other factors to consider before choosing an indwelling feeding tube for a particular animal include the etiology and location of gastrointestinal pathology, the anticipated duration of nutritional support, any risks associated with general anesthesia, the potential for hemorrhage or other surgical complications, and the level of animal and owner cooperation needed. However, as long as the gastrointestinal tract is functional, enteral nutrition has few disadvantages in most cats and dogs. A decision-making algorithm for the available routes of nutritional support in cats and dogs is shown in Figure 16-6.

The optimal diet for a cat or dog with cancer and an indwelling feeding tube is chosen after considering caloric distribution and nutrient content as previously described, with the added requirement that the ration be of a consistency that will pass easily through the tube. Small diameter tubes such as nasoesophageal and enterostomy tubes perform best when used with complete and balanced canned liquid products specifically formulated for tube feeding of cats and dogs (see Table 16-8). It is important to recognize that human liquid enteral feeding formulas are not complete and balanced for long-term use in small animals and tend to be low in both protein and fat and relatively high in carbohydrate calories. They are often inadequate with respect to their vitamin and mineral content as well. Paste-type critical care products (see Table 16-8) or blenderized canned cat or dog foods can be used with larger bore pharyngostomy, esophagostomy, and gastrostomy tubes since they are less likely to become clogged.
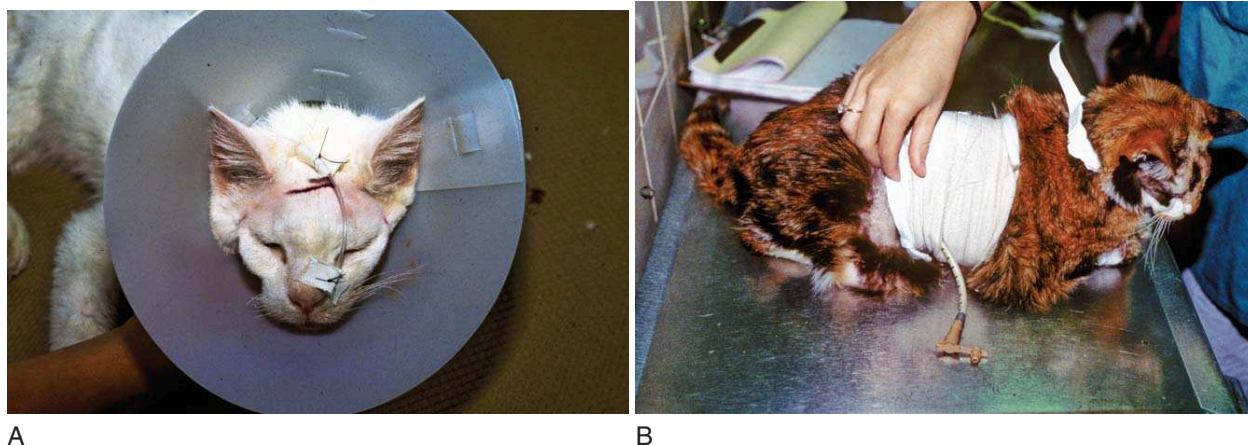
Figure 16-5

A, A cat with a nasoesophageal tube. This type of enteral feeding tube is most appropriate for short-term nutritional support. **B**, A cat with a gastrostomy tube. This type of enteral feeding tube can be left in place long term and can be used with paste-type critical care rations or pet food gruels.

The full target volume that is needed to meet the animal's daily energy requirements is calculated before feedings are initiated. The number of calories required over a 24-hour period is divided by the caloric density of the ration to be fed, giving a target quantity of food in milliliters, grams, cups, or cans:

$$\text{Food quantity} = \text{Total energy requirement (kcals)} \div \text{caloric density (kcal/mL*)}$$
$$= \text{mL* over 24 hours}$$

Bolus feeding is used with most tubes but should be started slowly, especially in chronically debilitated animals or those with underlying gastrointestinal dysfunction. Dilution of the feeding formula with water may help to decrease gastrointestinal side effects such as vomiting or diarrhea. If the initial regimen is well tolerated, the concentration and volume can be gradually increased over a period of days until the animal receives the full volume of food needed in 4 to 6 daily feedings. Continuous rate infusions are used with enterostomy tubes, with a gradual increase in the rate of infusion over 48 to 72 hours until the target volume is reached. Stable animals with feeding tubes can usually be managed by the owner at home, unless an enterostomy tube is being used. Critically ill cats and dogs with cancer should be hospitalized during the initial phases of feeding so that they can be monitored closely, regardless of tube type. Physical status and clinical pathology parameters including packed cell volume, total solids,

blood glucose concentration, serum potassium and phosphorus concentrations, and renal function should be followed closely for the first 24 to 96 hours.[31] Refeeding syndrome, which is characterized by profound hypophosphatemia, hypokalemia, glucose intolerance, and hemolysis, has been reported in cats and dogs receiving both enteral and parenteral feeding.[31,154,155] Cardiovascular collapse and death are possible in severe cases. This complication is more likely in severely malnourished animals, especially if they receive a high proportion of calories as carbohydrate. Refeeding syndrome is prevented by ensuring that severely depleted cats and dogs receiving assisted feeding are refed slowly, have adequate phosphorus intake, and are monitored carefully. Despite these measures, aggressive intravenous phosphorus supplementation is necessary in some cases to keep the serum phosphorus concentration within the normal range.

Enteral feedings can be stopped when the animal is capable of meeting its nutritional requirements through voluntary intake. Premature tube removal is avoided by discontinuing feedings but leaving the tube in place initially. Actual voluntary intake is measured for at least 24 hours. If voluntary intake is inadequate, tube feeding can be resumed; if it is sufficient to meet the animal's needs, the tube is removed.

Difficult ethical issues may be associated with the placement of feeding tubes in cats, dogs, and people with terminal cancer.[11,156,157] Prolonged survival with concurrently improved quality of life should be the clear goal in all cases; unfortunately, painful prolongation of the process of dying can be the result in some individuals. Percutaneous endoscopic gastrostomy

*May substitute grams, cups, or cans.
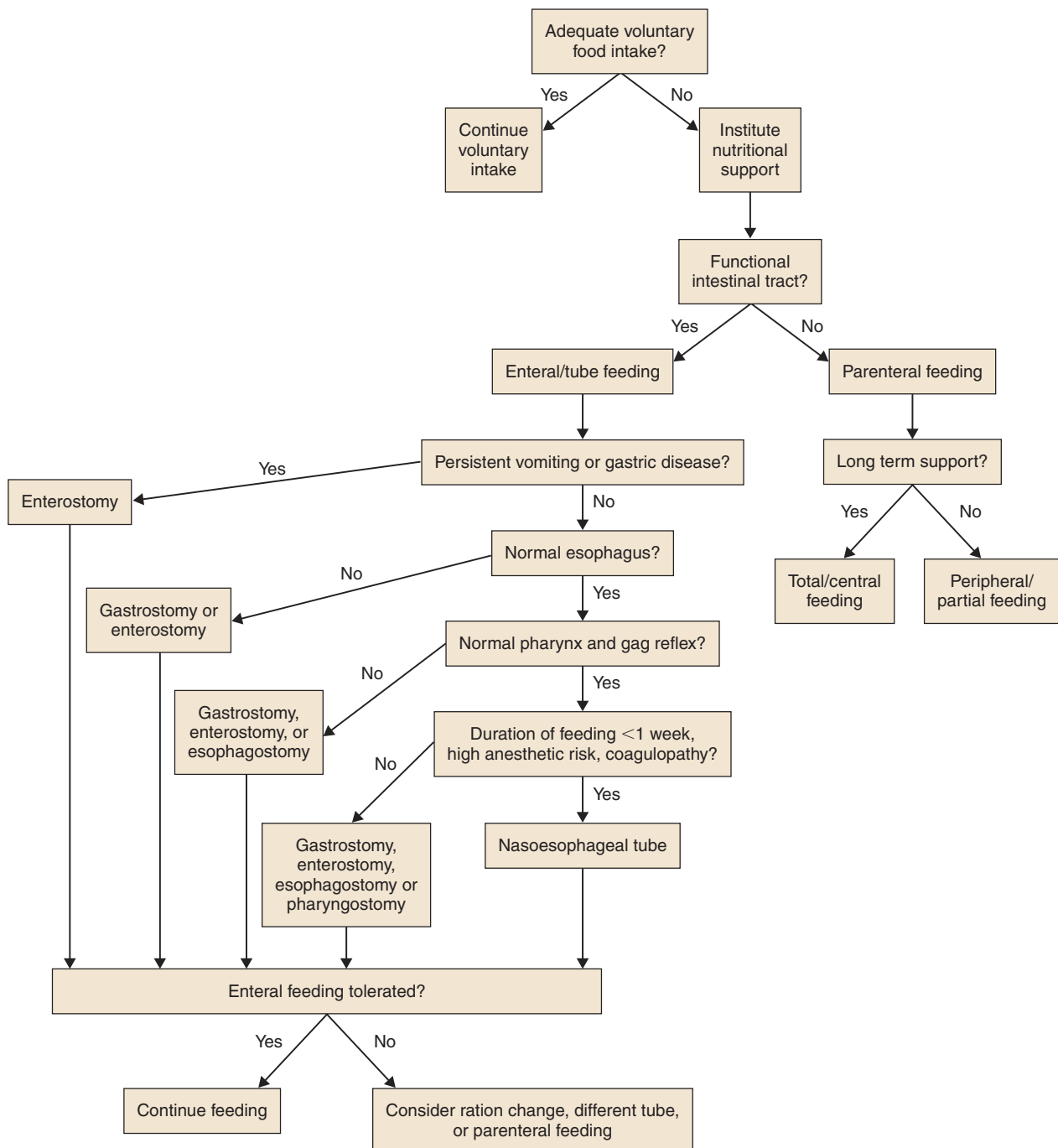
Figure 16-6

Decision-making algorithm for nutritional support. *(Adapted from Slatter, D., Textbook of Small Animal Surgery. Philadelphia, W.B. Saunders, 2003, p. 95.)*

(PEG) tube insertion does not improve nutritional or functional status in people with cancer who have a very short life expectancy.[157] Other factors that are associated with a negative outcome in people that receive PEG tubes are advanced age, severe underlying disease, diabetes mellitus, poor body condition with significantly decreased lean body mass, urinary tract infection, previous aspiration, serum albumin concentrations less than 3.0 g/dL, and hospitalization in a general medical center.[158] Extrapolation of these findings suggests that

cats and dogs with cancer should be carefully evaluated based on similar criteria prior to insertion of any type of feeding tube. A frank and clear discussion with the pet owner regarding the risks and benefits of assisted feeding is an essential part of this decision-making process. Assisted feeding should be a humane choice that is associated with real benefit in every animal that receives it.[159]

Assisted Parenteral Feeding. Assisted parenteral feeding is indicated in a relatively small subset of cats, dogs, and people with cancer.[2,6,11,138] It is the only alternative for nutritional support if the gastrointestinal tract is nonfunctional and represents an option for animals with obstruction caused either by tumor or severe ileus. Parenteral feeding is also used in the treatment of inflammatory intestinal diseases such as pancreatitis or inflammatory bowel disease, because it allows complete bowel rest. Parenteral nutrition can be administered safely in comatose animals because the likelihood of aspiration is decreased. Finally, parenteral feeding can be considered when there is a significant risk associated with general anesthesia or surgical placement of a feeding tube secondary to hemodynamic instability or coagulopathy.[30,31]

Parenteral feeding has several important disadvantages and potential complications, so it should be chosen as the method of nutritional support for the cat or dog with cancer only when it is clearly indicated. With the exception of persons undergoing bone marrow transplantation, studies examining outcome in people with cancer that receive parenteral nutrition have documented increased complication rates, equivocal improvement in measures of nutritional assessment, and trends toward decreased survival.[1,6,138] The lack of ingesta within the intestinal tract during parenteral feeding can lead to intestinal mucosal atrophy and compromised gut function within a matter of days. Bacterial translocation from the gut is also more likely in parenterally fed animals and people. For these reasons, a combination of parenteral nutrition and enteral support should always be considered for animals that will tolerate at least some enteral feeding. Even if only small volumes of food can be delivered into the gut, this will result in a significant improvement in intestinal function and immunity. Other complications that may be seen during parenteral feeding include mechanical problems with catheter function and intravenous lines; thrombophlebitis; biochemical abnormalities such as hyperglycemia, hyperlipidemia, and refeeding syndrome; and sepsis. Parenteral nutrition is relatively expensive and can be complicated to administer, requiring specialized equipment and expert nursing care. It tends to be impractical when the anticipated duration of support is less than 3 to 5 days.[30,31]

Parenteral feeding can be used to meet all nutritional requirements (total parenteral nutrition) or just selected essential requirements (partial parenteral nutrition).

The specific steps involved in designing, compounding, and administering a parenteral feeding prescription are discussed in detail elsewhere,[30,31,160] but the same basic guidelines regarding optimal calorie distribution and micronutrient content for cats and dogs with cancer that are fed enterally are followed. "Total" parenteral nutrition is the appropriate choice if long-term parenteral feeding is necessary, although this type of support is attempted much less often in animals than it is in people. Partial parenteral nutrition prescriptions may not be nutritionally complete by strict definition, but they supply adequate quantities of all essential nutrients for the short-term feeding that is typically used in small animals.[161-164] Total parenteral nutrition prescriptions are more complex and costly, and some expertise is necessary to prevent contamination and avoid mixture of incompatible solutions during compounding. Total parenteral nutrition solutions may also be hypertonic because of high dextrose content and should be administered into a large central vein to avoid thrombophlebitis. In contrast, the lower dextrose content and relatively decreased tonicity of partial parenteral nutrition solutions allows them to be administered peripherally. This may be the only option for animals in which parenteral feeding is indicated but central venous access cannot be maintained.[30,31]

Role of Nutrition in the Pathogenesis of Cancer

There is increasing evidence that nutrition plays a role in the pathogenesis of many types of cancer. These complex relationships have been best studied in people and rodent models. For instance, diets high in fiber have a protective effect with regard to colon carcinoma in people and diets high in saturated fats are believed to increase the risk of breast cancers.[147]

Very few studies have examined the role of diet in the pathogenesis of cancer in cats and dogs. One case control study investigated the effect of body condition and diet on the risk of mammary cancer in dogs. While the authors were unable to prove that a diet high in fat or an obese body condition 1 year prior to diagnosis was associated with an increased risk of mammary tumor, they did find that the risk of breast cancer was decreased in both spayed and unspayed dogs that had been thin at 9 to 12 months of age.[165] Another case-control study in dogs with mammary cancer also found that obesity at 12 months of age was associated with an increased tumor risk.[166] This study implicated habitual consumption of homemade rations and relatively decreased serum retinol concentrations in the development of canine mammary tumors as well. Finally, these investigators were able to show that dogs with breast cancers and dysplasias were more likely to have high intake of red meat, suggesting a possible role for fat

intake in the pathogenesis of these diseases.[166] In addition to promoting obesity, high-fat intake could lead to increased exposure to carcinogens since many of these compounds are fat soluble and would be contained in the adipose tissue of animals in contact with them. Animals consuming these fats as part of their diet would in turn have increased carcinogen exposure as well.[167]

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SECTION C
Pawspice: An End-of-Life Care Program for Terminal Patients

Alice E. Villalobos

PAWSPICE

The bond between pets and their human caregivers is a celebrated and cherished relationship. When it is time for the physical departure of the pet, the bond grows even stronger. Pet owners express their emotions and suffer anticipatory grief. They often want to spend as many last days with their pet at home as possible before the inevitable loss. Although this textbook is filled with a variety of ways to treat cancer more effectively, our patients remain at risk of death and often eventually do succumb. Cancer claims half of our senior pets, while organ failure concurrently deteriorates them as they age. Therefore, we often see simultaneous debilitating disorders in our cancer patients. Having more than one disorder in addition to cancer is associated with shortened patient survival times. With all this in mind, it's time to rethink how our profession can improve end-of-life services for terminal patients and help pet caregivers face the final days with their pet.

Most pet owners and their veterinarians have preconceived notions about cancer and its treatment. Obvious biases and ingrained feelings regarding cancer may cause a negative approach toward its treatment in young and middle-aged pets and more so in geriatric animals. Case by case, veterinarians and their professional staff must overcome the defeatist attitude about cancer therapy and end-of-life supportive care. We can dispel the old ways of thinking and the negative notions and myths that no longer serve us. Instead, the veterinary profession can propose an end-of-life hospice (Pawspice) care program for every client's pet. People come to us for advice and present their pets to us as patients. They do not expect us to prematurely terminate their pet without offering reasonable options. Our profession can, with solidarity, offer pet owners supportive, palliative options for complete care and attention to their pet's special needs when they are traveling down that final road toward death.

If the pet's illness cannot be treated due to financial constraints or a logistical problem, it is still a matter of good professional service to compassionately provide advice and meticulous home-care instructions. When a treated pet's cancer has repeatedly recurred, metastasized, is resistant to treatment, or if the pet is in the terminal stages of cancer, in home hospice (Pawspice) care is a next step that keeps both pet and caregiver comfortably close to their nest. A well-conceptualized,

creative, palliative Pawspice plan for pet owners may be the very best care that our profession can offer a terminal patient to support the people-pet bond.

Veterinarians and their staff can kindly and respectfully help sustain a quality life for terminal patients during the very last days and hours when the people-pet connection must be physically severed. They can help support the emotional needs of the ones that must say good-bye to their highly bonded pet. They can also uplift the grieving pet owners to focus on communication with their pet and to value the good parts of each day. In addition, they can help the pet owners to realize that the pet is still very much alive and with them during the final days and weeks.

Keep in mind that the pet's caregivers are also bonded to you and your staff and to the idea of coming into visit your facility and interacting with you on an intimate fashion. All this social contact with purpose is going to end abruptly with the passing of the pet. Pawspice is more than a farewell; it is a parting of connections and supportive relationships with you and your staff and a loss for the enormity of the care-giving routine.

When a pet is injured, gets sick, gets old, or has arthritis, degenerative myelopathy, or cancer, the question of home care always comes up. How much care is a pet owner willing to provide for an ailing pet? From this author's experience with treating cancer patients in California for 35 years, it can be stated that the answer to this question is very personal. For some pet owners, this special kind of willingness to provide end of life care may have no limits. Each pet owner has his or her own unique personal lifestyle and tolerance considerations. After an initial consultation with the primary veterinarian and a thorough consultation with a specialist regarding options for curative treatment or palliation, the owner(s) will struggle with decision making. Pet owners must weigh issues in their personal finances, the logistics of getting to and from the treatment center, and scheduling for visits and home care requirements. They must also face their reevaluation of their attachments for the pet in its compromised status. They need to deal with their feelings of guilt, sense of duty, and remorse as their pet encounters problems and their disability progresses. How they feel about participating in a treatment protocol while their pet is in reasonable health may be different after the pet encounters side effects from treatment.

Many pet owners don't even realize that they may be cheating themselves out of enjoyment with their pet because they are so upset over its impending death. They don't know how to deal with their anticipatory grief over the pending loss. Based on where they are coming from, their marital and family situations and other issues that are going on in their life, pet owners may respond with a wide range of emotions from panic all the way to shut down.

All pet owners weigh their specific issues. They either accept the challenge to treat their pet's illness with your recommendations or they decline. When they decline your plan, the battle is not over. Many pet owners seek the palliative therapy that may not have been wholeheartedly offered at your practice. Many others will sneak away from your hospital and pursue alternative treatments without your knowledge or guidance because they don't want to hurt your feelings. They are embarrassed or believe you will be angry with them or that you may disapprove. It is crucial that veterinarians refrain from being judgmental at this sensitive time. It is kind and proper to continue educating and offering options that may be more palatable for the owner to incorporate into their routine. You may have to adjust your offers of medical support toward the pet's maintenance level where you are only able to provide their pet comfort and support. If you create a protocol for this type of care, it becomes a very palatable end-of-life option and you keep your client's confidence.

It is also important to refrain from suggesting or insisting on euthanasia as the next best option if the pet is not treated at the high level you had initially recommended. Let the pet owner know that pain relief and the means to make the pet more comfortable are important to you and readily available at your facility while they are trying to make a decision regarding therapy. Many clients have complained to me that their initial veterinarian was too quick to recommend euthanasia if their pet's disease was incurable or because their senior pet had other concurrent conditions or was too old. Others complained that their veterinarian seemed to insist that the recommended procedures be done as an "all or none" policy. Some clients complained that they felt pressured to comply with procedure A or B, even though the pet's overall prognosis was poor. They complained that their veterinarian did not give them much of a choice, and if procedure A or B were not followed, their pet would suffer and needed to be euthanatized. Yet somehow, the consultation ended and their pet was discharged from the clinic without being offered adequate pain control and supportive care for the pet's ongoing condition at home. They felt abandoned, unsupported, and disappointed.

Clinical Case

Emma Moon, a 14-year-old F/S German shepherd mix, was the only pet of a childless, vibrant film business couple. One day, a very upset man entered our busy reception room. He asked our receptionist, Jennifer, if we had time to give a second opinion. Mr. Moon and his wife were told to euthanize Emma that day by two different specialists. Both an internist and a surgeon told them that Emma had two kinds of cancer, was very old, and needed to be euthanized. Jennifer comforted him

and told him that she would ask me to see if we could do anything to help. Mr. Moon sat on the floor with tears running down his face. I asked him to tell me what was going on. He managed to tell me that Emma was diagnosed with bone cancer a few months ago and that she had rapidly developed a tumor in her groin. It was diagnosed as mast cell cancer. He said that today's x-ray of her diseased leg looked like the bone cancer would fracture. Both specialists agreed that Emma should be put to sleep ASAP. Mr. Moon said that he and his wife were tormented over this advice. They were not ready to let her go because Emma still wanted to eat and be with them. The clients in our waiting room and my staff empathized with his distress. I emphatically told him, "Your dog does not have to die from euthanasia today if you are not ready." I told him that we could enter Emma in a palliative Pawspice program. I said, "If you are this conflicted and not ready yet, then it is not right for you." We told him to bring his wife and Emma and all the medications she was on, so we could evaluate her at the end of the day.

The couple returned at dusk carrying Emma on a big soft pad into an exam room. Emma's paws never touched the ground. She had pink mucous membranes and a pleasant interested look on her face. Mrs. Moon was puffy eyed and they both looked at me with hope. We examined Emma. She was breathing comfortably and had no heart murmur. She had a large swelling of her left humerus that had been diagnosed as osteosarcoma. Referral x-rays showed a large osteosarcomatous lesion with lysis and erosion of cortical bone. The lesion appeared to have a hairline fracture present but there was no displacement. A huge, bright pink soft tissue mass extended along Emma's left caudal mammary chain. The mass was $16 \times 8 \times 8$ cm. Fine needle aspiration cytology had diagnosed mast cell tumor earlier that day. Emma's abdomen was normal on palpation with no organomegaly. The couple said, "Look at her, she isn't ready to die yet, she still gets up to do her business and she eats and wags her tail and likes being at the center of things. We want help to keep her with us until we feel she is ready to go."

I explained to the Moon's that "Dogs and cats don't exhibit their pain in ways that are completely understood. Dogs by their very nature want to stay with their pack and will 'fake' wellness despite their pain. That is why a pet's pain may not be appreciated by their caregivers, even though they think that they know their own pet very well." I defended Emma's doctors regarding their opinion and verified the fact that Emma indeed had pain. The sheer volume and location of the mast cell tumor warranted a poor prognosis for any dog. Emma's mast cell cancer added yet another negative factor to the bone cancer. The pathological fracture, the fact she was on NSAIDs for the bone cancer yet needed steroids for the mast cell tumor (two medications that

cannot be safely combined), compounded with her advanced age, all added up to a very poor prognosis for Emma. I had no disagreement with her doctors' opinions yet could not disagree with the Moons. Emma was bright, alert, and responsive and she seemed to be quite content. We offered palliative Pawspice care. I said, "Look at Emma with your questions, and she will tell you in some way when the time is right."

We designed a special home care calendar with scheduled preemptive pain control for the bone tumor and palliative treatment for the mast cell cancer. We started Emma on fentanyl pain patches for osteosarcoma pain control. We administered dexamethasone and vinblastine for the mast cell cancer along with famotidine and benadryl injections, subcutaneous (SQ) fluids with vitamins B, C, and B_{12}. We reviewed the calendar schedule with its specific instructions for oral prednisone, chlorambucil, benadryl, and famotidine along with supportive supplements from our chemoprevention and immunonutrition protocol. The Moons were instructed to discontinue all NSAIDs and recheck with us weekly for vinblastine injections and to assess Emma's quality of life. To our amazement, Emma's mast cell tumor regressed completely. She survived with a good quality of life in her loving home for an additional 8 months in Pawspice care.

Reluctance and Fear of Needles and Tubes

Some pet owners cringe at the sight of needles or the thought of using a feeding tube, and many feel that they cannot handle the thought of giving injections. The idea of using a feeding tube may sound like heroics to one person and make perfect sense to another. We talk about feeding tubes being used routinely now, especially for cats, to provide them with proper nutrition during recovery from lipidosis, or after oral surgery, or during radiation treatments to the oral cavity. Many Pawspice patients need SQ fluids to maintain hydration, especially if the pet has concurrent renal disease, hypercalcemia, or other electrolyte imbalances. It is truly important to always speak to the Pawspice caregiver in a tender, unhurried fashion because that person is most likely under a tremendous amount of personal, financial, and emotional stress.

We approach the fear of needles with cheerful teaching. First, I ask the owner to look at my pen. Its function is to transfer information from me to the chart. I tell them that the syringe with its needle is like a pen, that it transfers medications from us to the pet. Consider the syringe to be a tool like a pen and nothing to be afraid of. We give demonstrations using saline. We ask the pet owner to actually give mock injections at home until they acquire the skill of handling needles and using syringes.

We try to lighten the demonstrations a bit and make suggestions for the mock injections (such as injecting teriyaki sauce into chicken) so the owners can have some fun.

Home Care for the Patient

Many pet owners want to nurse their terminal cancer pet at home. They need their veterinarian and the nursing staff to teach them how to care for their pet and maintain the finer points of hygiene and physical maintenance while working with their own unique inhibitions and concerns. Home care for a pet with incontinence or paresis is a task certain pet owners have chosen to tackle with the help of pet wheels, hoisting straps, harnesses, ramps, extra soft bedding, egg crate mattresses, and loads of towels and diapers. Some pets will need frequent bandage changes. Some will need to have their bladders expressed or will need diapers. Incontinent pets will need frequent bathing to avoid urine scald and fecal contamination.

Some pet owners feel that it makes perfect sense to acquire portable oxygen tanks in order to administer oxygen therapy for their pets with compromised respiration. Some people get upset with a growing tumor, thinking it might rupture and cause the pet to die. We comfort them and tell them that this would be unusual and that at worst, the wound might drain and discharge. Some people react with a paralyzing fear or nervousness at the sight of blood or oozing wounds (from radiation therapy mucositis and desquamation, ulcerated tumors, or decubital ulcers). Others cringe if they have to watch the nurses give injections or perform medical procedures. I solve this problem with frequent rechecks or hospitalization. Arrange technician or doctor house calls to assist the owner for a reasonable desensitization period until they can get a home care routine established. Other pet owners have great fortitude and interest in learning how to administer injections and medications and nursing care to their pets. They want this for the pet's convenience. Frequently pet owners are motivated to do home care for the financial savings.

Willingness: The Most Important Ingredient for Pawspice Programs

The most important ingredient to look for in oneself, in the hospital staff, and in the pet owner is willingness. Hold a staff meeting and ask the question, "Is being a compassionate veterinary care giving facility with a Pawspice program in agreement with the nursing and reception staff?" Put one special staff member as the appointed "Pawspice Support" person for a certain pet and its owner. Ask the client to direct phone calls and concerns to their designated staff member or to members of the clinic's cancer care support team.

Nursing staff can handle most of the phone calls regarding home care problems. Appointments should be made for the doctor to see the Pawspice pet on regular rechecks to answer major questions such as changes in prescription medications and quality-of-life assessment.

Philosophy of Comfort to the End

The nation is very impressed with the concern and care that the wellness and hospice communities across America have provided for people who are sick and dying. Veterinarians can win the loyalty of their clients by emulating the philosophy of the hospice movement in honor of that special bond that connects people to their pets. Moving away from suggesting utilitarian, premature euthanasia will certainly help keep our profession more heartfelt, cherished, and celebrated in the eyes of the public. I often explain to pet owners who were offended by a knee-jerk suggestion of euthanasia for their pet, that we veterinarians inherited our profession from rural, ranching, and agricultural roots. Ranch and farm animals had a utilitarian function and when that function was over, they were "disposed of." Most hospital staff members with a few years of experience may recall the insensitive term *disposal*, which was used for handling the bodies of deceased patients in their work orders and bills. We can all revise the terms for this service with more sensitivity. *Disposal* has given way to terms such as *arrangements*, *group cremation*, *individual cremation*, or *burial*. I explain to the pet owner that companion animal medicine is relatively young. Our profession's founding philosophy and terminology was basically forged from a shared stewardship with livestock and epidemiology. Pet animal medicine emerged from our profession into its current status, but some of the old terminology and utilitarian concepts are still around and may be unintentionally hurtful.

Times have changed, and companion animal medicine has evolved into a high-tech medical delivery system. We can hope it won't go the direction of "The Mindless Machinery of Medicine," where hospitals keep the gears rolling along insensibly to keep patients alive who are beyond hope. Bioethicists hope that our companion animal profession is able to cultivate sensitivity and keep a courteous caring balance with life and death as long as the patient has what we must define and defend as a good quality of life.

Unlike the hospice movement for people, the veterinary profession likely cannot sustain the time and personnel for fund-raising efforts or national organizations. We probably won't be organized and funded sufficiently to send registered veterinary technicians, nurses, bereavement counselors, beds, oxygen, meals, and wheelchairs for free. However, from exam room consultations, veterinarians and staff can gracefully provide the education and support needed for the efficient home care of ailing pets on a fee-for-service basis. Pet owners will gladly pay for the education that facilitates the control of pain and the art of providing good nutritional and hydration support for their pet. These consultation and demonstration sessions give the caregiver ability to care with expertise. This option gives pet owners more time to let go of their pet slowly and carefully. Many tender private moments of quiet emotion and sweet conversation are often shared between the caregiver and the dying pet.

Education of Home Caregivers

The most important factors to educate home caregivers to confidently monitor are quality of life (see the Quality-of-Life Scale section that follows), pain, nutrition, hydration, respiration, and sepsis. With training from the veterinary staff, home care may be almost like being in the hospital yet in obvious ways, better than being in the hospital. Ask the question, "If this pet owner were trained to take the pet's temperature, administer subcutaneous fluids and necessary injections, and provide the proper pain control, oral medications, sufficient food, and hygiene, can this pet have some well being and live longer at home?"

Quality-of-Life Scale Helps Decision Making

There is a need for assessing various levels of quality of life for aging, ailing, and terminally ill pets and especially the cancer patients under our care. Most companion animals have one or more abnormal conditions that appeared in their senior years and these conditions generally worsen with time. One third of our pet population is overweight and/or obese. When these pets become burdened with cancer and its related treatment issues, their preexisting conditions complicate matters or may limit their owner's choice of options for treatment. Veterinarians are frequently asked, "When is the right time to euthanize my beloved pet? How will I know?" I always tell the pet owner that " One day it will gel and you will just know it is the right time because your pet will tell you with a look or a gesture, a sign, or a series of bad days." A quality-of-life scale may help everyone, especially those in denial, to look at issues that are difficult to face. Caretakers can use this itemized scale to ask themselves if they are able to provide enough help to maintain an ailing pet in the proper fashion and cleanliness.

Every animal has certain needs and desires that should be recognized and respected. If we can meet these basic needs and desires at a satisfactory level for our ailing companion animals, then we are justified in preserving the life of the ill pet during its steady decline toward death. The goal in setting up the quality-of-life

scale is to provide an easy guideline for assessment of the pet so that pet owners can maintain a rewarding relationship that nurtures the human animal bond. This quality-of-life scale will relieve guilt feelings and engender the support of the veterinary team to actively help in the care and decision making for end-of-life, Pawspice patients.

The basic needs and desires that are innate to the quality of life for sick pets should not and cannot in good consciousness be ignored. It is up to the veterinary profession and to the pet's individual caretaker to design an end-of-life program (Pawspice) or pet hospice that encounters each factor and deals with it openly and honestly with an achievement of quality-of-life scale from 1 to 10; 10 is the best.

Below is a proposed list of the basic desires that need both healer and caretaker's attention. I have christened this scale with the name "HHHHHMM," so it is easy to remember the pet's needs and desires that we feel every Pawspice program should strive to satisfy: *hurt, hunger, hydration, hygiene, happiness, mobility*, and *more* good days than bad days. A score above 5 on most of these quality-of-life issues is acceptable in maintaining a pet hospice, Pawspice, or end-of-life care program. Each pet's situation needs to be customized, and each pet owner needs to be recognized as an individual who needs a kind, supportive coaching approach to come to terms with the decision to end a best friend's life.

The HHHHHMM quality-of-life scale

Hurt: 0-10. No hurt. Adequate pain control is first and foremost on the scale. This includes the pet's ability to breathe properly. Most people do not realize that not being able to breathe is ranked at the top of the pain scale in human medicine. Attention to the pet's ability to breathe properly is a top priority. Cases with pulmonary effusion need thoracentesis on an as-needed basis. Pet owners need to be trained to monitor the pet's respirations and comfort level and to identify labored breathing so they won't wait too long to provide relief. Some families are willing to provide oxygen therapy at home for their ailing pets. The veterinarian can prescribe oxygen through a medical supply house. Pain control may include oral, transdermal, and injectable medications and be given preemptively.

Hunger: 0-10. No hunger. If adequate nutrition is not being taken in by the pet willingly or by hand, coaxing, or force feeding, then placement of a feeding tube needs to be considered. Cats do very well with esophageal or gastrostomy feeding tubes. Malnutrition develops quickly in sick animals when the caretaker is not educated enough to know how much his or her pet needs to eat to maintain body weight. Instruct owners to use a blender or liquid diets to help their pet maintain proper nutritional and caloric intake. Many pets will live much longer if offered wholesome, flavorful foods that are varied. My own Great Pyrenees, Alaska,

went from a life of consuming only dry food to canned food to hamburger, fresh baked turkey, to chicken, to various types of sausages, to venison, pastrami, cheeses of all types, and gourmet cut and marinated meats. She liked parmesan cheese, smoke flavor, alfredo sauce, and cheddar cheese soup mixed into her food along with lots of encouragement, coaxing, and hand feeding. It takes patience and gentle concentrated coaxing to get some Pawspice pets to eat. It is hard not to be disappointed when such specially prepared food is rejected. Just come back with another offering with a different flavor a little later, and the patient may find the meal more appealing.

Hydration: 0-10. No hydration problems. Educate the pet owner about adequate fluid intake per pound (10 ml per pound per day) and to assess for hydration by the skin pinch method. Subcutaneous (SQ) fluids are a wonderful way to supplement the fluid intake of ailing pets. It may take a few demonstration sessions for a pet owner to learn how to administer SQ fluids. This helpful procedure saves the client a lot of money and keeps the pet on a much healthier status. Subcutaneous fluid ports can also be placed in a short and simple surgical procedure. Giving SQ fluids can make a huge difference in quality of life during Pawspice.

Hygiene: 0-10. Can the pet be kept brushed and cleaned? Is the coat matted? Is the pet situated properly so that it won't have to lie in its own excrement after eliminations? Pawspice pets, especially cats with oral cancer, can't keep themselves clean, so they may get demoralized quickly. The odor associated with necrotic, oral tumors can be offensive and cause social rejection by family members. Instruct the pet owner to use antibiotics to help reduce foul-smelling infections. Dampen a sponge with a very dilute solution of lemon juice and hydrogen peroxide and gently stroke the face, paws, and legs of the patient. This action is similar to a "mother tongue" and helps to clean the fur while soothing the unkempt cat. Dogs love this type of facial and paw grooming too!

Happiness: 0-10. Is the pet able to experience any joy or mental stimulation? It is easy to see that our pets communicate with their eyes. They know what is going on via their senses and mental telepathy. Is the ailing pet willing to interact with the family and be responsive to things going on around him? Is the aging cat able to purr and enjoy being on the bed or in one's lap? Is there a response to a bit of catnip? Can the cat bat at toys or look and follow a laser light? Can the ailing pet enjoy the upbeat greetings and petting of loving family members? Can the pet's bed be moved close to the family's activities and not left in an isolated or neglected area? Is the pet depressed, lonely, anxious, bored, or afraid? Do you have a routine fun time that the pet looks forward to?

Mobility: 0-10. Ask if the pet is able to move around enough on its own or with help in order to satisfy

its desires. Does the pet feel like going out for a walk? Is the pet showing CNS signs, seizures, or stumbling? Can the pet be taken outdoors or helped into the litter box to eliminate? Will a harness, a sling, or a cart be helpful? Is medication helping?

The answer to the mobility question has viable and variable scenarios, and the scale score is acceptable anywhere from 0 to 10. I have met some pet owners who are, in my thinking, too rigid for their requirements in mobility of their pets. For instance, they are regretfully but willing to sacrifice their pet's life rather than elect amputation of a limb. Some pet owners have the honest yet teleological feeling that amputation is mutilation and not fair to the pet. Instead, they allow the pet to bear a painful limb for months before euthanasia. Then there are cases like 12-year-old, male, 90-pound golden retriever Krash Pancino of Orange County. Krash's mobility was already borderline when he entered our Pawspice program with osteosarcoma of his left distal radius. His history precluded amputation because of severe degenerative joint and disk disease and degenerative myelitis. Krash had severe osteoarthritis of both his knees along with moderate to severe degenerative joint disease (DJD) from hip dysplasia. Krash wore a splint when he went to the park to offset a potential pathological fracture.

In my opinion, the mobility scale can be variable from 0 to 10. The need for mobility seems dependent on the species and breed. Cats and small lap dogs can and do enjoy life with much less need for mobility than large and giant breed dogs. If the pet is compromised and is only able to lie in bed, is there a schedule to change the position of the pet and rotate the body at least as often as every 2 hours? Can the pet's bed be moved around the house to keep the pet entertained and in the family's company? Atelectasis of the lungs and decubital ulcers must be avoided. The nursing care of large immobile dogs is very demanding. Is the bedding material soft enough? Can an egg crate mattress be used and set up properly to avoid decubital ulcers? Is there a role for a pet mobility cart or an Evans standing cart? These items really make a difference in the quality of life for the pet that has limited mobility yet is still alert and responsive.

More Good Days Than Bad Days: 0-10. Ask if there are more good days than bad days. When there are too many bad days in a row (or if the pet seems to be "turned off" to life), the quality of life is too compromised. Bad days are those filled with undesirable experiences such as vomiting, nausea, diarrhea, frustration, falling down, or seizures. Bad days could be from a condition that worsens such as cancer cachexia or the profound weakness from anemia, from the discomfort caused by gradual tumor pressure or from obstruction or a large, inoperable tumor in the abdomen. This was the situation with my own dear 11½-year-old Australian shepherd, Alfie. He had a huge, undifferentiated mass that rapidly expanded his liver. If the two-way exchange needed to communicate

and maintain a healthy human-animal bond is just not there, the pet owner must reconcile or be gently told that the end may be near.

It is very difficult for families to make the final decision to end a beloved pet's life by euthanasia. Coming to a decision about euthanasia is especially avoided when euthanasia is against the pet owner's religious beliefs. Sometimes they are not sure about the quality-of-life issues at the very end. It can be made clearer to them if the standard scale for quality of life is set ahead of time and reevaluated every couple of weeks or every few days or hours as the situation requires. If the pet is slowly passing on with a peaceful tranquility, then that may be a satisfactory situation. People often really want their pet to pass on naturally at home in their arms or in their own bed. That is okay as long as the pet is just weakening steadily and not suffering. We as veterinarians need to accept the fact that it is a personal and natural wish when our clients request us to help their pet to die naturally at home. We are the one's they turn to for help and we have an oath "to use our scientific knowledge and skills for the benefit of society through the protection of animal health and the relief of animal suffering." We can help our client's pet loss needs and at the same time guarantee that the pet has a pain free passage by using preemptive pain medications. Here is where a referral for home euthanasia with a kindly house call veterinarian should be recommended. We can hope that using this conceptualized user-friendly "HHHHHMM" scale for quality of life will facilitate the heart-wrenching decision that euthanasia truly is and that our professional guidance can help relieve the angst and regret about a beloved pet's death that often haunts pet owners for the rest of their lives.

Satisfaction: An Important Ingredient for Pawspice Care

Ask the pet owner if he or she is satisfied with the Pawspice arrangement. At times, there is conflict in the family about how attached the main caregiver is to the pet. Some family members feel inconvenienced with the soiling, the odors, the sacrifice, the sadness, or the diverted attention and devotion of the caregiver. This issue of disapproval creates a double-edged sword for the patient's main caregiver to deal with on a daily basis. I like to address this issue directly with the family so they can come to a supportive consensus. If this issue goes beyond your personal counseling ability, suggest a family counselor.

It is also important to determine with yourself, as the doctor, and with your professional staff if it feels rewarding or at least satisfactory to help preserve the bond between this person and this pet. If these positive ingredients are present, then pet hospice or Pawspice is a good experience for all involved.

For specifics on pain and nutritional management, the reader is referred to other sections of this chapter. Technician house calls may be set up for those who are unable to deal with administering injections and fluids to the Pawspice pet. The reception staff can be made aware of which pets are on Pawspice programs so they can be sensitive when family members call in for information, appointments, or emotional help.

Pet loss group sessions can be helpful for the most affected individuals. We held weekly pet-loss group meetings at our hospital on Saturdays. Pet owners found them rewarding because they could share their feelings with others. Locate a pet-loss support group that you can refer your clients to if they need extra help with coping.

Helpful Home Care Books

People crave information about their pets using the Internet and available books. It is handy to have copies of the following book in your lending library: *Pets Living with Cancer: A Pet Owner's Resource* by Robin Downing, D.V.M., available from AAHA Press, Lakewood, Colorado.

Networking by phone or online is also helpful. Give the phone number or the e-mail address of another client who is providing the same type of Pawspice care so they can have the opportunity to share their experience with the newcomer. Of course, ensure the other client has consented to the use of their e-mail as a resource.

Hospital Day Care

The primary care veterinarian's facility can conveniently provide daily help for Pawspice pets while the owners are at work. This service may include taking the pet's temperature and weight, bathing and brushing the soiled pet, SQ fluids, injections, and hand feedings. This service can be the key to sustaining a quality Pawspice for the working pet owner. Convenient monthly billing and drop-off and pickup times that revolve around the owner's schedule may be prearranged with attending staff members before and after routine receiving hours.

Sir Walter Scott's Question

Sir Walter Scott wrote an insightful short poem that showed how much he appreciated the human-animal bond. I like to read it out loud to my patient's caregivers when they enter their pet into a Pawspice program or when we are talking about euthanasia. The poem is featured in *Angel Pawprints* by Laurel Hunt. With one changed word (dog to pet), this poem expands to include cats and other species of pets.

"I have sometimes thought of the final cause of dogs [pets] having such short lives and I am quite satisfied it is in compassion to the human race; for if we suffer so much in loving a dog [pet] after an acquaintance of ten or twelve years, what would it be if they were to live double that time?"

SUGGESTED READINGS ▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪

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SECTION D
Bond-Centered Cancer Care: An Applied Approach to Euthanasia and Grief Support for Your Clients, Your Staff, and Yourself

Laurel Lagoni

"Knowledge of the biologic behavior and methods of treatment alone will not make one an effective clinical oncologist. However, the replacement of an overwhelming need for CURING with an attitude of CARING is a positive first step."[1]
—Steven E. Crow, DVM

In 1997, my family and I agreed to appear on a segment of ABC Television's *20/20* program. The producers were filming a piece on pet loss with Colorado State University's Animal Cancer Center, and our family dog Toby was dying of lymphoma. Twice, over a 3-month period, my two young daughters, my husband, and I met with the segment's producer and crew as they documented Toby's treatment and decline. Finally, when we knew the time was right, I called the producer's New York City office, and they returned to our home with Bob Brown (their on camera interviewer) to tape Toby's last day and her euthanasia.

Why would I agree to do this? Because, at that time, I had spent the last 15 years of my professional life providing grief support for pet owners and grief education for veterinary students at Colorado State University's

James L. Voss Veterinary Teaching Hospital, and I had an agenda. As the co-founder and co-director of Changes: The Support for People and Pets Program (now known as the Argus Institute for Families and Veterinary Medicine), I had listened to and learned from thousands of veterinarians and pet owners who had experienced the deaths of their patients and beloved companion animals due to cancer. Now, I had the chance to tell people the three most important things I had learned about the disease:

1. A diagnosis of cancer in a pet is not an immediate death sentence.
2. Being present with a pet when he or she is euthanized is not only permissible but also comforting, especially for children.
3. Many veterinarians are skilled at providing both high-quality medical care for animals *and* high-quality emotional care for people—and these veterinarians are worth searching out.

The program aired in November 1997, and I still get calls and letters from people telling me how much it meant to them to actually see my family there, on the floor of the hospital's client comfort room, surrounding our dog, hugging her and sobbing our eyes out, as she died. It also meant a lot for them to see the veterinarian and staff comforting us. Even though clients tell me it was sad and hard to watch, the program gave them the courage to be present with their own pets when they were euthanized. It also prompted them to seek out veterinarians—especially when their pets were diagnosed with cancer—who were as sensitive and caring as the ones who helped my family.

The veterinarians who watched the program told me it helped them feel better about this most dreaded and difficult part of their jobs. They realized that by skillfully conducting euthanasia with respect and sensitivity, they could feel they were still succeeding with their clients—providing comfort—even though there was no longer much they could do medically to help their patients. For them, providing skilled emotional support became as much a priority as providing medical treatment or palliative care.

As a veterinary oncologist, you are trained to take a rational, scientific approach to case management. Your concerns are accurate diagnosis, effective treatment, and, ultimately, cure of the disease. You are probably quite comfortable providing complex information and using state-of-the-art technology. However, when it comes to providing emotional support for your patients' families, particularly when their pets die, you may not feel so confident.

Just as with medical expertise, confidence about comforting others grows with an increase in knowledge and skills. This section of the chapter provides you with a working knowledge of family-present euthanasia and grief support and helps you feel more prepared to provide comfort for grieving clients and stressed-out staff.

COMFORT AS A TREATMENT GOAL

In 1998, Dr. Franklin McMillan suggested in a commentary in the *Journal of the American Veterinary Medical Association (JAVMA)* that comfort, not health, is the "primary and central objective of medical practice."[2] Dr. McMillan proposed that disease is fought for the purpose of providing and maintaining comfort, thus, health professionals should view the elimination of disease as the single most important part of eliminating discomfort. "Medical care is an intervention, the outcome of which is patient [and client] comfort," stated Dr. Franklin.[2]

Studies have found that relief of distress is the leading reason for seeking medical care.[3] The effort to minimize psychobiologic distress is often described in the medical literature as the act of providing comfort.[4] Comfort is defined in various ways, but most often is described as a physical, mental, or emotional state of ease or well-being. The word *comfort* means "to strengthen" and is derived from the Latin word *confortare*.[5]

In the old paradigm of veterinary medicine, providing comfort for patients and clients was viewed as a fallback position, the behavior to use when medical treatment for a pet was less than successful. Providing emotional support or comfort for clients was regarded as a service ancillary to the delivery of medical care and was most often associated with the work of nurses and other members of a veterinarian's support staff. In many clinics, these beliefs and role assignments led to a separation between the functions of providing client comfort and treating patient illness or injury. "This dichotomy is false," stated Dr. McMillan, "as health and comfort are as inseparable as disease and discomfort, which are strongly related."[2]

In the new paradigm of veterinary medicine, and especially in veterinary medical oncology, providing comfort for both patients and clients is as much a priority as providing medical treatment. As a contemporary veterinary oncologist, you understand that it is no longer acceptable or effective to simply treat *bodies*. Today, you must also care for the *bonds* that exist between patients and their human families, as well as those that develop between clients and your entire veterinary oncology team.

BOND-CENTERED CANCER CARE

Bond-centered care refers to veterinary services that simultaneously address the medical needs of patients

and the emotional needs of pet owners.[6] Bond-centered care considers the relationship, or bond, that exists between a pet and the animal's family and allows that relationship to influence the progression of treatment, as well as the circumstances surrounding the pet's death.

When the bond-centered care approach is used, decisions and behaviors are *not* totally driven by the animal's physical needs. For example, when you practice bond-centered care, you may agree to keep a dying animal alive for a few hours (or days) more than what your best medical judgment tells you to do in order to allow an absent family member time to travel home to say goodbye to their pet in person. As another example, you might allow clients to decorate their hospitalized pet's cage with cards and family photographs. Even though these items might be a bit in your way as you monitor your patient, you understand that the gesture helps your clients feel more connected and supportive of their pet.

Using the bond-Centered Care approach, you concurrently assess, support, and respond to the emotional needs of people, as well as to the medical needs of pets (Box 16-1). Your clients' emotional needs often include the following:
- A sensitively delivered diagnosis
- Openness and patience during repeated questions
- Access to both medical and mental health professionals who sincerely listen to their concerns
- Support and reassurance during open displays of emotion
- Assistance during decision making
- Skilled crisis intervention and management
- A humane euthanasia technique that is also designed to provide emotional catharsis and support when families are present
- Grief education and support before, during, and after their beloved pet's death

Box 16-1	**The Emotional S.O.A.P.** **A Case Management Model for Providing Emotional Support**

Medical variable		**Emotional variables**

• How do you think this animal is doing?
  ➢ physical appearance
  ➢ body language and demeanor
  ➢ interactions with the owner
• What is the reason for this visit?
• What does your intuition tell you about this patient?
	$S\|S$
	Subjective
	What do you feel/notice/suspect?
		• How do you think this owner is doing?
		  ➢ physical appearance
		  ➢ body language and demeanor
		  ➢ interactions with the pet
		• What might the owner need from you?
		• What does your intuition tell you about this owner?

• What does the owner tell you about this animal and the presenting problem? • What is the important medical history? • What do you find on physical exam?	$O\|O$ Objective *What are the facts?*	• What does the owner tell you about his/her feelings and relationship with this pet? • What is the important emotional history? • What do you find on the Family-Pet Relationship Information Form?
• What past experiences and knowledge can you draw on for this case? • What diagnosis can you rule in based on your collected information?	$A\|A$ Assessment *What can you conclude from an overall synthesis of the data?*	• What past experiences and knowledge can you draw on for this case? • What emotional needs and support-based services can you rule in as potentially applicable to this case?
• What options can you recommend and offer for treatment? • What is the time frame for treatment? • What is the cost of treatment? • What is the treatment follow-up?	$P\|P$ Plan *What treatment and support options are available to owners?*	• What options/resources (supportive people, finances, time) are available to this owner? • What is the time frame for support? • What is the cost of the recommended support services? • What is the support follow-up?

If you and your oncology team members are committed to attending to, rather than overlooking, most of these client needs, you are providing bond-centered cancer care.

Why Is Being Bond Centered So Important?

Since the 1980s, personal and societal values and beliefs concerning the human-animal bond have changed significantly and, in turn, "the bond" has significantly changed the practice of veterinary medicine. Today, pet owners know it is acceptable and healthy to grieve when their pets die, and they expect their veterinarians to help them through this grief.[6]

To meet your clients' expectations regarding grief support, it's helpful to understand why the human-animal bond has become a more significant force in your clients' lives. It's also helpful to understand the normal grief process. In addition, it's important to develop an effective technique for conducting family-present euthanasia and to have some knowledge about dealing with the workplace stress caused by patient death.

The Human-Animal Bond

Societal changes, as well as medical advances, over the past several decades have caused the bonds between people and pets to intensify. Today there are more divorced, widowed, never-married, and childless people in Western society than ever before. In addition, family mobility away from relatives and close friends, along with self-care situations for children of working parents, are common. This lack of consistent human support and companionship has caused pets to become a greater daily source of emotional and social support for many children and adults. Research tells us that 85% of pet owners consider their pets to be members of their families,[7] with 70% of pet owners describing their pets as children.[8]

Researchers who study the bonds we share with companion animals have discovered many benefits to forming relationships with pets. These include lowered heart rates, blood pressure, and stress levels from petting, or even just watching, animals.[9] Pets have also been linked to the development of self-esteem and empathy in young children,[10] as well as the alleviation of loneliness in the elderly.[11]

Recently, Dr. Johannes Odendaal found that interactions between dogs and humans lowered blood pressure and concentrations of cortisol, while increasing concentrations of dopamine, endorphins, oxytocin, and other neurochemicals associated with the physiology of positive interactions.[12] There is mounting evidence that interactions with animals can have positive affects on human health and psychosocial well-being.

Yet just as there are many benefits to these bonds, there are also consequences. One of the most difficult consequences of bonding with companion animals is the grief that is experienced when companion animals die.

When the Bond Breaks: Grief

Pet death is an inevitable part of pet ownership and a predictable part of veterinary medicine. Studies show that the emotions pet owners feel when their pets die are often overwhelming and that the grief response to pet loss is often on par with the grief response to a human loss.

In one study, researchers examined the grief responses of more than 200 middle-aged couples who reported the death of a pet within the past 3 years. Of 48 life events, including the death of a spouse, divorce, marriage, loss of children, an arrest, loss of a job, and the death of a pet, researchers found that the death of a pet was the most frequently reported trauma experienced by the couples participating in the study. Survey participants said the death of a pet was less stressful than the death of a human member of their immediate family but more stressful than the death of other relatives. Forty percent of wives and 28% of husbands reported that the loss of a pet was "quite" or "extremely" disturbing.[13]

Normal grief

When the human-animal bond is broken by death, both owners and veterinarians experience loss. Loss is defined as an ending or as a point of change and transition.[14] Grief is the natural and spontaneous response to loss.[14]

Grief is the normal way to adjust to endings and change and a necessary process for healing emotional wounds. Grief is a *process*, not an event. The progression of normal grief happens over varied and individualized time frames. Depending on the circumstances and significance of the loss, normal grief may last for days, weeks, months, or even years.

The grief response is unique to each individual. *There is no right or wrong way to grieve*. Grief is unique to different groups, societies, and cultures. In most cases, the variables of age, gender, and developmental status greatly affect peoples' expressions of grief. For instance, research confirms that women shed more tears and cry more often during grief than men.[15] This is probably due to the fact that men are socialized to maintain their composure during emotional times, while women are socially conditioned to express their feelings more openly.

Children also grieve just as deeply as adults.[6] Due to their shorter attention spans, though, they do so more sporadically. Children most often express their grief through behaviors rather than through words. They act out their grief through artwork, play behaviors, or expressions of anger and irritability (Box 16-2). This is due, in large part, to the fact that until children reach the ages

Box 16-2	Suggested Emotional Support Protocols for Veterinary Oncology

Six ways to help children with pet loss

The death of a family pet is often a child's first experience with grief. Pet loss can be a valuable time to teach children how to deal with grief in healthy ways.

1. *Be direct and honest.* Avoid euphemisms like "put to sleep" as these terms can be confusing and even frightening for young children.
2. *Tell the truth about the circumstances of the pet's death.* If parents or veterinarians lie to children to "soften the blow," they risk substituting one kind of pain for another. When uncovered, lies can damage trust between children and parents, as well as between children and veterinarians.
3. *Involve children in their pet's treatment and euthanasia.* Children are often involved in their pet's daily routines and care. They should also be included in discussions and decisions regarding their pet's death.
4. *Allow children to be present during euthanasia.* When children are well informed and prepared, they can usually make their own decisions about whether or not to be present during euthanasia. If children choose not to attend, they should be encouraged to say good-bye to their pet and to view their pet's body after death has occurred.
5. *Encourage children to memorialize their pets.* Some children write poems about their pets or plan actual funerals. Parents can frame a favorite photo of the pet or create a short videotape of the pet that children can watch as often as they want.
6. *Discourage immediate replacement of pets.* When a special pet dies, it is important to take time to grieve. Some families adopt a new pet immediately, believing that a new relationship will distract children from the negative feelings of grief. This strategy can backfire, though, if children fail to bond with the new pet due to an incomplete grief process.

Box 16-3	Symptoms of Grief

Physical
Crying, sobbing, shock, a lump in the throat, shortness of breath, stomachache or nausea, tightness in the chest, restlessness, fatigue, sleep and appetite disturbance, body aches

Intellectual
Denial, sense of unreality, confusion, inability to concentrate, hallucinations concerning the loss (visual, auditory, and olfactory,) a need to reminisce about the loved one and to talk about the circumstances of the loss

Emotional
Sadness, anger, depression, guilt, anxiety, relief, loneliness, irritability, feelings of being overwhelmed or out of control, feelings of hopelessness and helplessness, feelings of victimization

Social
Feelings of withdrawal or a greater dependency on others, a desire to relocate or move, a need to find distractions from the intensity of grief (to stay busy or to overcommit to activities)

Spiritual
Renewed or shaken religious beliefs, searching for a meaningful interpretation of a loved one's death, paranormal visions or dreams concerning a dead loved one, the need to "finish business" with a purposeful ending or closure to the relationship (a funeral, memorial service, last rites ceremony, good-bye ritual)

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Adapted from Lagoni L, Butler C, and Hetts S, The Human-Animal Bond and Grief. Philadelphia, W.B. Saunders, 1994.

of 8 or 9, they do not possess the cognitive development and language capabilities necessary to verbally express grief.[16]

Clinical experience shows that when the expression and emotional experience of grief is restricted in some way, the healing time for recovery is prolonged and the outcome is more negative. Likewise, when grief is freely expressed, the healing time for recovery from loss is, in general, greatly reduced and the grief outcome is more positive.[6] In your role as veterinary oncologist, you can be instrumental in creating a positive grief outcome for your clients. As Dr. Steven Crow said, "Responding to grief in pet owners is an important measure of a veterinarian's humaneness."[1]

Anticipatory grief

Along with normal grief, it is important for veterinary oncologists to understand the concept of anticipatory grief. Anticipatory grief occurs prior to an actual death and often begins with diagnosis. As soon as you say the word *cancer*, most pet owners sense that they may potentially lose their relationship with their companion animal and they may exhibit any or all of the manifestations of normal grief detailed in Box 16-3.

As pets' conditions deteriorate, owners adjust to the changes that have occurred in their pets' appearances, personalities, and physical capabilities due to treatments or surgeries. As they adjust, many experience a sense of loss as they give up knowing their pets in the ways they used to know them. During this period of anticipatory grief, pet owners begin the process of

saying good-bye to their pets, with some consciously or subconsciously detaching from their pets (and perhaps rushing the decision to euthanize) and others clinging even more tightly to the relationship (and perhaps prolonging the decision about euthanasia for too long).

Anticipatory grief is often characterized by anxiety, worry, guilt, confusion, and indecision. Sometimes clients who are in the midst of anticipatory grief are misunderstood and thought of as difficult or 'problem' clients. These are the clients who ask unending questions, question your competency, exhibit peculiar behaviors, or demand unreasonable access to you or to their hospitalized pets. However, "problem clients" are often grieving clients and can usually be stabilized with honest conversations about grief and sincere emotional support.[6]

As a veterinary oncologist, you can best help all of your grieving clients by acknowledging the special bonds they share with their companion animals, validating the emotional distress that often accompanies medical information and decision making, encouraging your clients to openly express the normal symptoms commonly manifested by grief, and learning to assess and refer clients who are in need of more intensive grief counseling to qualified mental health professionals. While it is unrealistic to expect you to provide long-term support and follow-up for grieving clients, high-quality cancer care includes the ability to identify clients who may be at higher risk level than others for a negative grief outcome. It also requires you to be familiar with the local community resources that are capable of addressing your client's psycho-emotional needs.

Noted grief expert Murray Colin Parkes said, "It is important for those who attempt to help the bereaved to know what is normal."[17] Developing a comfort level with normal grief leads you to feel more confident about helping your clients manage the symptoms of grief. You can think of this as providing emotional palliative care for your grieving clients. Box 16-3 provides you with a holistic overview of the normal symptoms of grief.

EUTHANASIA

Veterinary medicine has always accepted the responsibility of euthanasia, believing it is a privilege to help animals die in a humane, painless way. Researchers and clinicians have invested considerable time into the development of drug recommendations and clinical protocols to guide veterinary teams through the medical aspects of euthanasia.

Since the 1990s, protocols have also been developed to guide veterinary teams through the emotional aspects of euthanasia, teaching veterinarians and their staff members how to cope with their clients' (as well as their own) feelings of grief. These protocols are designed to

guide veterinary teams through dozens of common situations related to client communication, companion animal death, grief support, and stress management.[18,19] The focus for the remainder of this chapter describes the bond-centered cancer care approach to (1) the processes of preparing families to face their pets' euthanasias, (2) the sensitive facilitation of family-present euthanasia, and (3) effective follow-up care after euthanasia.

Preparing for Euthanasia

When it comes to preparing your clients to cope with the painful feelings surrounding their pets' deaths, a pre-euthanasia consultation is a necessary and effective emotional support protocol. The goal of this consultation is to educate your clients about end-of-life issues and to gain consensus about several aspects of the euthanasia procedure. This consultation might take place rather spontaneously during a telephone call, a regularly scheduled examination or treatment appointment, or it might be a scheduled meeting designed for this specific conversation. (*Note:* With the bond-centered care approach, providing comfort and end-of-life consultation is a valuable service and a legitimate use of your professional knowledge and time. Therefore, you should charge clients accordingly).

The key concept to keep in mind during a pre-euthanasia consultation is *choice*. When people feel they have choices, they feel more in control and able to make decisions that are right for them. Research shows that the freedom to choose, as well as a longer preparation time (anticipatory grief), diminishes the intensity of grief once a loved one has died and aids the emotional healing process.[17,20] For this reason, it is important for clients to have a thorough understanding of their pet's prognosis and to have as much time as possible to plan and prepare for the death.

Pre-euthanasia consultations are most productive when held face to face, in a quiet, private place where clients will not feel hurried or pressured to make decisions. Many veterinary oncology practices have created client comfort rooms (Figure 16-7) where these conversations, as well as euthanasia or visits with hospitalized patients, can take place. During this conversation, you should expect to encounter a wide range of emotion (e.g., sadness, anger, guilt, frustration, and even relief). You should also be prepared to support these emotions by having tissues, water, and other comfort items readily available in the room.

Entire families should be encouraged to attend a pre-euthanasia consultation. If your clients have children, this is an excellent time to ensure that they understand their pet's condition and that they will be included in how the family decides to say good-bye. During the consultation, you can discuss several topics with your

Figure 16-7

A comfort room can be clinic space dedicated solely to euthanasia procedures and patient visits or an exam room that is quickly and easily converted for nonmedical consultations.

Figure 16-8

Large floor mats allow families and pets to be comfortable together during euthanasia or during visits with a hospitalized patient. Comfort mats can be custom made to fit your exam rooms and can hang on the wall behind a door when they are not in use.

clients and help the family make as many decisions as possible prior to their pet's death. The following topics should be discussed.

Timing

Clients use many methods to decide when the time is right to euthanize their pets. Some let the medical signs point the way, while others ignore the medical evidence and allow themselves to be guided solely by their emotions. Almost every discussion about when to consider euthanasia, though, centers on the pet's quality of life. A helpful way to measure quality of life is to compare the pet's normal, healthy behaviors and personality with the pet's present experience of life.

Although it can be emotionally difficult, once your clients have realized that euthanasia is imminent, it is most beneficial to schedule an appointment time for their pet's euthanasia. An appointment is the only way to ensure that a beloved pet will die in the way in which they have chosen, with you and everyone else who wishes to be there able to attend.

Location

The site for a family-present euthanasia might be outdoors, at the family's home, or in your specially equipped comfort room inside the veterinary hospital. When your staff knows where the family wants to say their final good-bye to their pet, the staff can prepare the site ahead of time. For example, if the family prefers to say their last good-byes outdoors, tissues, grief education materials, and some type of ground covering can be placed in the space prior to their arrival (Figure 16-8).

Body care

Owners should be offered all of the options, including necropsy, that are available to them in your community, and each should be explained with honesty and sensitivity. The cost of each option should also be disclosed. It is helpful to use visual aids during this explanation. For example, if your practice makes cremation available, sample urns and cremains can be available to view if clients choose to see them.

Memorializing

Families can be encouraged to plan ways to pay tribute to their pet, both prior to and after death. Many pet owners want to spend special time with their pets prior to euthanasia, taking pictures or making videotapes. Others want to make plans for a postdeath funeral or memorial service. Memorials help bring meaning to loss and help families draw closure to relationships. However, without prompting from you, these helpful strategies may not occur to clients and may cause them to feel great regret once their pet has died.

Family presence

Clients of all ages need to decide for themselves whether or not to be with their pets when they die. Presence at death is often important for owners as they want to "be there" for their pet just as their pet has always "been there" for them. Even though euthanasia is emotional to witness, research tells us that emotional catharsis and support from others during grief are two primary elements of creating positive grief outcomes.[21] Both occur during well-planned family-present euthanasia.

To decide whether or not to be present, families—including young children—need honest, detailed information from you regarding your preferred protocols for euthanasia. During the pre-euthanasia consultation, detailed information about euthanasia as a medical procedure should be provided. Points to cover include the drugs that are used, use of a catheter, possible side effects of the drugs, and the time frame within which death will occur. Detailed information about the emotional aspects of the euthanasia procedure should also be provided. For instance, clients should be told that they will be encouraged to pet, talk to, and spend time with their pet before, during, and after their pet's death.

Facilitating Family-Present Euthanasia

With the exception of medical emergencies, families who have a scheduled appointment for their pet's euthanasia should be given first priority over everything else that is happening at that time in your clinic. Upon their arrival, your clients should be met at the door and immediately escorted by you or a staff member to wherever the euthanasia will take place. Once they are settled, the euthanasia consent form should be signed, arrangements for payment should be made, and the pet should be removed to a treatment area so a catheter can be placed in the animal's hind leg.

The placement of a catheter is always a point of debate when discussing family-present euthanasia. Many veterinarians insist that a catheter is not necessary in terms of their own medical skill and the cost it adds to the procedure. Clinical experience with euthanasia would probably support this belief. However, the use of a catheter can enhance the emotional experience for clients, eliminating the possibility that a pet will flinch, cry out, or appear to struggle away from the needle. Any one of these behaviors are usually enough to change a comforting experience with euthanasia into a disturbing one for your clients.

When the family has been offered time to spend alone with their pet (not all families will want this) and is ready to proceed with euthanasia, you can gather everyone around your patient, with you at the animal's feet with access to the catheter and your clients at the pet's head, with access to the animal's face and front paws. Once everyone agrees that it is time to begin, you should make each injection quickly, with little or no lapse of time between them, naming them as you go, so clients understand how the procedure is progressing. For example, you might say, "First, I'm injecting the saline flush to make sure the catheter I have inserted is working properly…. Now I am giving Pepper a barbiturate that will make him sleepy and relaxed … Now I am injecting the final drug, the one that will stop Pepper's heart." Aside from these statements, it is best to remain silent. Most owners want to focus on saying good-bye to

their animals and find comments, questions, and chatter distracting to this profound, private experience.

Once the procedure is completed, it is very important to use a stethoscope, listen for a final heartbeat, and to pronounce the animal dead. A clear, simple statement such as "Pepper has died" is most effective. If you try to soften the blow with euphemisms like, "Pepper is gone," clients are often unsure that death has actually occurred. Your statement will often trigger sobbing and even feelings of relief. Remember, expressions of grief are normal and even healthy. Owners may make remarks about how quickly death came and about how peaceful the experience was. Some will express feelings of guilt and others will tell stories, reviewing the pet's life. This is a good time for you to reassure your clients about their decision to euthanize and to express your own feelings of affection and grief for your patient. It is also a good time to offer families the chance to clip some of their pet's fur or make a clay imprint of their pet's paw (Figure 16-9). Keepsakes like these often become meaningful links to pets in the days and weeks of adjusting to life without their longtime companions.

Before a pet's body is removed for cremation or necropsy, clients should be offered more time alone with their pet. If they are ready to leave, a member of your staff should stay with the body while you walk your clients outside to their car. Almost all owners take one last look back at their pet before they actually leave the euthanasia site. When they see their pet accompanied by a friendly, familiar face, they feel reassured that their companion animal will not be forgotten or treated with disrespect once they leave.

Exiting through a back door is preferable for clients so they don't have to face others who may be in your waiting room. If clients are taking their pet's body with them for burial, they should be asked to bring a box, blanket, or other acceptable container with them to the

Figure 16-9

An imprint of a pet's paw creates a meaningful keepsake.

procedure. Before the body is moved, clients should be reminded that body waste and fluids may leak out and that it may be helpful to wrap the lower part of their pet's body in plastic. Finally, you should always take care to handle a dead pet's body with great care and respect. Sometimes the most efficient way to move a body can be disturbing for a client to witness. If you must move a body in an awkward way, prepare your client for what they may see or ask them if they would like to step out of the room until the process is completed.

After-Death Follow-Up

In veterinary oncology, the strong bonds you form with your clients don't diminish with your patients' deaths. During treatment and the dying process, clients have learned to trust you and your judgment. It is logical that they will rely on that trust and look to you for guidance as they grieve.

For the majority of clients, the condolence card, memorial gift, telephone call, or flowers you send will be enough to comfort them and to reassure them that you are available should they feel they need to reconnect with you. In addition, you should provide every client with written information about grief and the names and contact information for several pet loss counselors, hot lines, or websites.

A small percentage of your clients are more at risk for a negative grief outcome and may require more active support from you and your staff. These clients would benefit from an additional follow-up telephone call, during which time you can assess their progression through grief and make another attempt to refer them for further counseling. At-risk clients might include those who:
- live alone
- were deeply attached to and emotionally dependent on their pet
- have little or no social support
- express a great deal of guilt and regret
- have experienced other recent losses
- have a history of mental illness
- have made suicidal comments
- seem to be stuck in the grieving process, unable to recover

Referrals often need to be made several times before clients actually act on them, so remain consistent with your suggestion. In general, it helps if you have personal knowledge about the counselor or program to whom you refer, but that is not always possible. Look for programs associated with universities or professional organizations and check out counselors' credentials and experience. Without formal training in grief support and *a sensitivity to pet loss*, counselors can do more damage than good.

Stress Due to Patient Death

Stress is the normal response to a demand. The demand may come from a major event (medical emergency) or from the cumulative effects of minor, everyday hassles (time crunches, noise, and staff miscommunication).

Stress can be positive or negative. Negative stress, or "dis-tress," is experienced when you must make adjustments to an unwanted demand like the death of a patient.

Stress is inevitable in veterinary practice. Much of it results from trying to balance high-quality care for dying pets and their owners with your own personal and professional needs and limitations.

Caregiver stress

You can expect your own grief to be present during patient death. Crying, feelings of sadness and relief, as well as confusion and fatigue are normal and should be expected. Research in human medicine, with subjects who are professional caregivers for dying patients, shows their grief reactions to be similar to those of the families they work with, although they are usually of lesser intensity and shorter duration.[22] Research and clinical experience also show that as professional caregivers increasingly attempt to respond to the needs of those who are dying, they form even stronger attachments to patients, leaving the caregivers even more vulnerable to experiencing grief.[23]

Some stresses are specifically related to helping others during times of loss and grief and are uniquely associated with the care of terminally ill patients and their families. Researchers Vachon,[24] Lazare,[25] Cook and Oltjenbruns,[26] and Peteet et al.[27] identified some of the common stressors that arise when working with the dying. With some adaptation to veterinary practice, those stressors are the following:
- Difficulty accepting the fact that the patients' physical and psychosocial problems cannot always be controlled, especially with "special" patients
- Frustration at having invested large amounts of energy in caring for a patient who then dies, taking this investment with them
- Disappointment if expectations for patients to die a "good death"—however this may be defined—are not met.
- Difficulty ending a life you once saved
- Difficulty establishing a sense of realistic limitations on what veterinary care, which is expected to be all encompassing, can actually provide
- Tension between co-workers who prioritize cure and remission versus those who are concerned about ethical issues like informed consent, medical experimentation, interference with a dying patient's comfort, and the determination of do not resuscitate (DNR) orders

- Difficulty deciding where to draw limits on involvement with patients and their owners, particularly during off-duty hours
- Feeling overwhelmed by the frequency of losses (sometimes several patients die within a short time span)
- Holding on to unresolved guilt concerning patient care, especially when a misdiagnosis or mistake is made
- Difficulty with the social negation of the loss (when the impact of the loss on the caregiver is not acknowledged by others)
- Belief in the assumption that one has to be strong because of a given occupation or role

Stress and euthanasia

Due to the option of euthanasia, it is estimated that veterinarians experience the firsthand deaths of their patients five times more often than their counterparts in human medicine.[28] Even when procedures go smoothly and clients are compassionately supported, the taking of a life remains a profound and, at times, a distressing process.

In a survey of veterinary students and practitioners conducted at the University of Georgia College of Veterinary Medicine, survey participants reported their physical and emotional reactions to performing euthanasia. Sixty-one percent felt a lump in their throat, 57% felt depressed, 48% felt anger, 46% felt like crying, 41% felt a sense of failure or inadequacy, and 35% felt the need for solitude.[29]

Small animal veterinarians at the University of California-Davis told researchers that frequent performance of euthanasia is one of the primary reasons for their burnout.[30] These veterinarians reported performing a median of eight euthanasias a month and commonly reported that they had not been prepared in college for dealing with euthanasia, but rather had learned a variety of strategies for dealing with euthanasia through their experiences.

To reduce the stress associated with euthanasia—for the client and for themselves—these veterinarians took great care when handling the animals before, during, and after euthanasia. They used gentle and respectful touches, tranquilized the animals, catheterized the veins, and, if owners had not been present during their pets death, the veterinarians presented the animals attractively. They reported that they avoided problems when conducting euthanasia by communicating clearly, using sensitive and comforting language, expressing compassion, acknowledging the client's feelings, allowing the client to be present during euthanasia, offering a soothing environment, and sharing experiences with colleagues.

Peak stressors were listed as accidental deaths in their hospitals, dealing with long-term clients' losses, and the needless euthanasia of healthy animals or those that could have been saved by veterinary care.[30]

Stress Management Strategies

Despite long work hours and high patient mortality rates, a study of physicians and their co-workers at a comprehensive human cancer care center reported a high incidence of job satisfaction.[27] Unlike those practicing other medical disciplines, oncologists seemed to enjoy more experienced team members, lower rates of staff turnover, and a greater appreciation of one another's skills. In addition, oncology teams reported that they shared the same goals, placing high priorities on emotional care, teaching, and physical care that was not necessarily focused on a cure. These were viewed as achievable goals and helped each team member feel successful and satisfied with his or her work.

Developing a strong, supportive team is one of the best ways to counter stress in veterinary oncology. When you trust the other members of your team, you are more likely to share responsibilities, create flexible work routines, and strike a healthy balance between your professional and personal lives. All of these factors have been identified as effective stress management techniques for oncology professionals.[27]

There are thousands of ways to cope with professional stress, yet some strategies are more relevant to dealing with patient loss and your own grief than others. Some of these strategies are discussed in the following sections.

Debriefing

Debriefing means talking openly and purposefully with your staff about the emotional aspects of your cases. The content of debriefing discussions might include new or challenging events in your practice, learning new information, sharing thoughts and feelings, and getting feedback and support for your actions.

Evaluating cases by debriefing allows you to acknowledge what was done well and what was not. It also encourages your veterinary staff to talk about their individual frustrations about not being able to cure or save particular animals.

In a good debriefing session, whether it is one on one or in a group, participants listen to and acknowledge the feelings of the speaker(s) without judgment. A facilitator uses open-ended questions to encourage speakers to continue and to help each staff member thoroughly understand the problem. Open-ended questions cannot be answered with a simple yes or no. Examples of open-ended questions are the following:

"What is it about this particular case that is bothering you?"

"What do you wish you could to do about it?"

"What ways have the rest of you found to handle situations like that?"

Keys to effective debriefing are as follows:

- Debrief on a regular basis, perhaps in a regular weekly rounds time. Regular debriefing helps prevent stress buildup and helps people build knowledge and skills.
- Meet in a private, quiet, comfortable room where you will not be interrupted. This environment encourages thoughtfulness and expressions of emotion.
- Be courageous and ask for feedback from your colleagues. On the other hand, offer feedback to others in an honest and sensitive way. Try to clarify your needs before the debriefing begins. For example, say, "I would like to hear from each of you about what you think I did well on this case and what you think I could do differently next time."
- Set time limits for the discussion before starting.

To deal effectively with the stress associated with grief, you need debriefing time to process information, share memories, and express personal feelings about your patients' illnesses and deaths. This is *essential* for avoiding burnout.

Drawing closure

Drawing closure means taking steps to intentionally bring cases to an emotional end. It means working hard to resolve nagging questions and to stop the "what ifs" and "if onlys" from invading your thoughts. When your cases are emotionally resolved, you realize that you did the best you could do at that time and understand what you would change or do differently next time in a similar situation.

Sometimes, all it takes to draw closure to a case is to send condolence cards to clients or to follow-up with them by phone. Other times, it requires more digging. For instance, you may need to track down leading-edge medical information or experts to better understand a patient's illness or unexplained death.

Clean time

Clean time is exactly what it sounds like. It is time that is free of professional demands and responsibilities. It is time that is not cluttered with the worries of work.

You should aim to find clean time on a daily basis, but it is especially important when your day has involved a lot of loss or emotional strain. During clean time, you might read, exercise, have dinner with friends (and not talk about work!), or watch a movie or television program. During longer periods of clean time, you might go fishing, take a dance class, volunteer, or travel.

It's important to ensure that your staff gets clean time, too. Allow them to take a "mental health day" once or twice a year. With mental health days, staff members call in "well" rather than "sick" and spend the day doing something nice for themselves. Mental health days can be scheduled a bit ahead of time out of courtesy for others. This kind of support for one another strengthens relationships in the clinic.

Help others

Helping relationships are based on respectful attitudes and an awareness that you are sharing the burden of problem solving, rather than fixing other peoples' problems. When you are helping others deal with emotions, it is not helpful to try to cheer them up or to distract them from their feelings. Rather, it is helpful to acknowledge their feelings, listen to their concerns, and guide them through their decision-making processes. Box 16-4 provides a list of suggested emotional support protocols to use when helping families through their pets' cancer treatment and eventual death. Box 16-5 describes how to achieve a "helper's high" for yourself as a benefit to providing comfort for others.

Seek professional emotional support

You may find that particular cases involving patient death or client grief touch you and cause you to react more strongly than others. Mental health professionals call this being "triggered" or "hooked" by cases. This is normal. It happens to everyone who works with people who are experiencing emotional pain. These are the cases, though, that you may want to look at more closely with the help of a grief counselor or therapist, asking questions like, "Does this client remind me of someone I have negative feelings about? Does the decision at hand remind me of a loss I have faced in my personal life?"

Some communities offer support groups for medical professionals through the local hospital, hospice program, or even veterinary association. Objective opinions from mental health professionals or even discussions with other trusted veterinarians may help you resolve conflicts about troubling cases. When selecting someone to consult with, do so with care. A nonjudgmental listener will provide support and validate your feelings without advice-giving.

CONCLUSION

It is important for veterinary schools, as well as practice owners, to prepare veterinary oncologists to provide care for patients, clients, and themselves with training programs that place a greater emphasis on palliative care, clinical communication skills, emotional support protocols, and stress management. These subjects are the realities of life. They cannot be avoided even with advanced medical skill and knowledge. A lack of balance

Box 16-4	Helping Families Cope with Cancer[32,33]

- Deliver every diagnosis in private using the three-step format recommended by crisis intervention experts.[1] (1) Prepare the client by saying something like, "Mary, I have something to tell you that will be difficult for you to hear." (2) Deliver the news using simple, common words like, "Pepper has cancer." (3) Pause briefly to allow clients to express emotions, then offer clients a choice about how to proceed, saying something like, "Mary, would you like me to explain the treatment options now or would like a few moments to call your family and take in this bad news?"
- Consider tape-recording each conversation about diagnostic results and treatment options so client can easily and accurately share the information with other family members. Research shows audiotapes reduce the number of calls for clarification back to a practice.[2]
- Offer at least three treatment options, including palliative care, standard treatment, and, when appropriate, euthanasia. Try to remain neutral by not recommending one option over the others.
- Encourage second opinions and referrals to hospitals where patients could become part of research studies and clinical trials.

- Prepare families for the emotional as well as the physical impact of each phase of treatment. Always offer the option to stop treatment.
- Encourage families to take photographs and to make videotapes of their pet's everyday routines—sleeping in their favorite spots, eating their favorite treats, playing with their favorite toys—before their pet's appearance changes drastically as cancer progresses to the end stage.
- Always discuss pain control for your patient and emotional support for your client. Make providing comfort a priority. The possibility that their pet is experiencing pain is a primary concern for most pet owners. This worry causes clients emotional discomfort.
- When treatment options fail, gently guide families to plan and prepare for the death or euthanasia as much ahead of time as possible. Remind clients that appointment times for euthanasia may seem unthinkable but allow you to arrange your schedule so you can be there when they need you.
- When it is time for euthanasia, give the entire family—including young children—the option to be present and to say good-bye in personally meaningful ways. Remember to offer linking objects like snips of fur or clay impressions of their pet's paw.

Box 16-5	How to Achieve a "Helper's High"[34]

Make personal contact
Connect with the person you are helping. Face-to-face support leaves a more long-lasting impression on you than a less personal task such as donating money or clothing to a nonprofit organization.

Help others frequently
Helpers who help others more frequently are the least likely to report health problems. The ideal frequency is about 2 hours per week.

Help a stranger
Helping strangers rather than family members is often viewed as a choice rather than an obligation. Choice brings more satisfaction to helpers.

Find a shared problem
Helpers who assist others with problems they themselves have experienced (e.g., alcoholism, cancer, poverty) are more likely to feel increased empathy for the person's struggle.

Work with an organization
Helpers report that when they feel a sense of teamwork and connection with other helpers, they are more likely to reach out and to help with regularity. Helping on your own is often more sporadic.

Use your skills
Your abilities should be appropriate to the level of challenge; otherwise attempts to help might produce negative results for all involved.

Let go of results
Health-enhancing emotions come from participating in the helping process itself, not from concentrating on producing certain outcomes or results.

between technology and humanism can diminish not only your patient/client care but your own sense of success and self-esteem.

As Dr. Martin Abelhoff of The Johns Hopkins Oncology Center said, "If we reaffirm that our top priority must be the well-being of our patients [and clients] and are courageous enough to examine critically our other priorities, we can begin to formulate solutions that will serve our patients [and clients] well and enhance our sense of professionalism and accomplishment."[31]

Dealing effectively with euthanasia and grief on the job takes practice. It takes commitment to confront emotions rather than walk away from them. It also takes dedication to ongoing skill building and stress management techniques.

When you possess the ability to provide effective emotional support (for yourself as well as for others), you feel confident that you and your staff can move through the stressful situations that you encounter on an almost-daily basis. When your emotional support and stress management protocols work, you are freer to do more of what you love—practice high-quality veterinary oncology.

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Complementary and Alternative Medicine for Patients with Cancer

Narda G. Robinson

The editors, Stephen J. Withrow and David M. Vail, wish to guide the reader to the website of the American Cancer Society relating to Complementary and Alternative Therapies (www.cancer.org/docroot/ETO/ETO_5.asp). In particular, the editors have added the following quote from the aforementioned document:

The American Cancer Society realizes that there are many definitions for the terms "alternative" and "complementary" methods and makes the following distinction between these categories. Alternative methods are defined as unproved or disproved methods, rather than evidence-based or proven methods to prevent, diagnose, and treat cancer. "Complementary" methods are defined as supportive methods used to complement evidence-based treatment. Complementary therapies do not replace mainstream cancer treatment and are not promoted to cure disease. Rather, they control symptoms and improve well being and quality of life. This distinction separates methods based on how they are promoted and used.

The American Cancer Society is sensitive to the growing public interest, in particular, those living with cancer, in information about alternative and complementary methods. The American Cancer Society acknowledges that more research is needed regarding the safety and effectiveness of many of these methods. The American Cancer Society advocates for peer-reviewed scientific evidence of the safety and efficacy of these methods. All cancer interventions must withstand the scrutiny of peer-reviewed scientific evaluation before they can be recommended for the prevention, diagnosis, or treatment of cancer.

The American Cancer Society realizes the need to balance access to alternative and complementary therapies while protecting patients against methods that might be harmful to them. The American Cancer Society supports patient access, but strongly encourages more oversight and accountability by governmental, public, and private entities to protect the public from harm as they seek therapies to complement mainstream cancer care. Harmful drug interactions may occur and must be recognized. Unnecessary delays and interruptions in standard therapies are detrimental to the success of cancer treatment.

The American Cancer Society supports the right of individuals with cancer to decide what treatment is best for them. But we encourage people to discuss all treatments they may be considering with their physician and other health care providers. We also encourage people with cancer to consider using methods that have been proved effective or those that are currently under study in clinical trials. We also encourage health care professionals to ask their patients about their use of alternative and complementary methods. Health care professionals should listen and know how to communicate with their patients. Open, trusting, non-critical dialogue is essential in this important area.

(Developed by the ACS Advisory Group on Complementary and Alternative Methods and reviewed by the ACS Medical Affairs Committee August 12, 1999. Reviewed by Harmon Eyre, MD, December 6, 2004.)

WHAT IS COMPLEMENTARY AND ALTERNATIVE MEDICINE (CAM)?

Complementary and alternative medicine embodies a diverse and sometimes controversial group of treatments that exist outside of mainstream medicine. Whether a given therapy is "complementary" or "alternative" depends on the practitioner's training, bias, and scope of practice. For example, animal cancer patients receiving acupuncture, manual therapy, or herbs in addition to chemotherapy, radiation therapy, or surgery for their cancer could conceivably enjoy the best of both worlds. In this instance, the acupuncture, manipulation, and herbal treatments would fulfill a complementary function alongside allopathic care, providing supportive means to soften the impact of conventional treatments and hasten recovery. One can also consider

the combination of conventional and complementary treatments as creating "integrative" care; the term integrative oncology is coming into vogue in cancer clinics such as the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York.[1]

In contrast, "alternative" medical practitioners eschew mainstream medicine and frequently advise clients to avoid standard treatments in favor of something else; typically, these treatments involve strict and unusual dietary regimens, fasting, enemas, or unusual chemicals. Several so-called alternative cancer "cures" are not merely unproven; they are actually disproved, according to research methodologist Andrew Vickers, PhD, at MSKCC.[2] Thus, practitioners espousing alternative options for animals with cancer may pose special risks to patients by substituting questionable therapies for standard, science-based treatments.

Compounding this problem is the growing number of individuals who are not veterinarians who are offering such alternatives. Their lack of veterinary medical training hinders their capacity to make medically sound recommendations for animals and to rapidly identify health problems and disease progression, should they arise. For cancer patients, delays in prompt and appropriate medical attention may make an otherwise treatable condition untreatable.[3]

Therefore, veterinarians need to (1) remain the primary care providers for animals and (2) be able to properly advise their clients on CAM and indicate which approaches are rational, beneficial, and safe. Veterinarians who either ignore or castigate their clients' interest in CAM lose meaningful opportunities for client education. Further, veterinarians unwilling to weigh the pros and cons of CAM with their clientele may unknowingly encourage them to turn to individuals who are not veterinarians or the Internet for options. This exposes patients to unnecessary risks, which one can circumvent by cultivating honest and open two-way communication.

Popularity

The popularity of CAM for cancer is undeniable. A 2005 study of human cancer patients by the James P. Wilmot Cancer Center at the University of Rochester indicated that 91% of patients surveyed reported using at least one form of CAM.[4] At the Animal Cancer Canter at Colorado State University, over 97% of clients claimed they had either a strong or average interest in CAM for their animals.[5] Despite this figure, 57% of clients confessed that they did not discuss their interest in CAM with their regular veterinarian.

Many CAM therapies once shunned by the medical establishment are now undergoing reevaluation and scientific testing. In 1998, the National Cancer Institute established the Office of Cancer Complementary and Alternative Medicine (OCCAM) to coordinate their research program in CAM.[6] In 1999, the American Society of Clinical Oncology and the American Cancer Society hosted their first-ever symposium on alternative and complementary medicine.[7] Several large drug companies are developing herbal and nutraceutical products obtainable over the counter; some of these are promoted specifically for the prevention of cancer.[8]

With information on CAM widely disseminated by the media and over the Internet, the range of assorted treatment possibilities available for use by veterinary clients seems infinite, and clients can obtain them without their veterinarian's consent or knowledge.

The purpose of this chapter is to present information on the mechanisms of action, side effects, and potential benefits of CAM approaches that are popular among veterinary clients for the treatment of cancer. However, nearly all of the studies done involve either humans or laboratory animals, so deciding which products and treatments are safe and effective for companion animal cancer patients will require further investigation. As such, this chapter is only intended to provide an overview. It is *not* a "how to" guide.

Botanical Medicine (Herbs)

Of all the antitumor and antimicrobial drugs either currently commercially available or in the late stages of development, 62% come from natural sources, such as plant products.[9] A study showed that almost 14% of veterinary oncology clients tried herbs for their animals with cancer, in hope of curing the cancer, reducing pain, preventing side effects, and improving general well-being.[5] Clients may neglect to mention the number and types of herbal products they are administering, either because they feel that their veterinarian does not know or does not care about their use. However, it is imperative that veterinarians scrutinize the list of botanicals clients are giving their animals not only to assess the products' inherent safety, but also to anticipate and thwart unforeseen drug-herb interactions. Some integrative oncologists advise altogether against co-administering herbs with chemotherapy, radiation, or surgery, citing concerns of interference with drug metabolism and coagulation.[1] Many common herbal products modulate intestinal P-glycoprotein or cytochrome P450 enzymes, producing clinically important herb-drug interactions.[10]

Still, herbal enthusiasts claim that safety concerns are overblown. They cite the World Health Organization as stating, "A guiding principle should be that if the product has been traditionally used without demonstrated harm no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment… Prolonged and apparently uneventful use of a substance usually offers testimony

of its safety."[11] Nonetheless, herbs are occasionally misidentified or contaminated, and in the case of certain Chinese herbs (Chinese patent medicines), herbal medicines sometimes contain pharmaceuticals not listed on the label.[12-15] Botanical medicines comprise an array of chemicals that vary in relative concentration depending on plant genetics, growing conditions, plant parts included, time of harvesting, preparation, and storage. Some herbs are themselves carcinogenic, such as comfrey (*Symphytum officinale*) which is hepatotoxic, and birthwort (*Aristolochia clematis*), which has been linked to urothelial malignancy. Nonetheless, even with the dramatic rise in the popularity and consumption of herbal products, there are few confirmed reports in which consumers have been harmed.

The paucity of research on herb safety in veterinary patients forces veterinarians to rely on anecdotal reports of their use in animals or extrapolate from other species. The Center for Veterinary Medicine (CVM) at the Food and Drug Administration (FDA) has stipulated that dietary supplements (which include herbs) need to undergo the same testing as new animal drugs. In their *FDA Consumer Magazine*, the agency declared that "CVM officials are concerned about these products because there are no scientific data showing that they are safe or even contain the ingredients listed on the label."[16] Of further concern is the absence of data regarding appropriate dosages for animals. As such, dietary supplements sold for animals without FDA approval are illegal, yet enforcing regulations is generally of low priority for the FDA CVM in most instances due to budgetary constraints.[17]

The herbs listed in Table 17-1 constitute only a partial listing of the many botanical agents with purported anticancer activity. Some of the herbs listed are individual plant compounds; others are mixtures.

Nutraceuticals

The term *nutraceutical*, coined in 1989, apposes the first part of the word *nutrition* with the last part of the word *pharmaceutical*.[151] It refers to "any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease."[154]

TABLE 17-1   Botanical Compounds with Purported Anticancer Activity*

Name and Description	Reported Effects in Cancer Cells or Model Systems	Adverse Effects and Precautions	Interactions with Other Herbs, Drugs, Foods, or Treatments
Aloe vera	Increases immune function. Stimulates production of interleukin-1 (IL-1) and tumor necrosis factor (TNF) by macrophages. May inhibit metastasis.[18]	Avoid in intestinal obstruction, pregnancy.[19] May cause diarrhea.[20]	Aloe vera gel applied topically along with hydrocortisone acetate may enhance antiinflammatory activity.[21]
Artemisinin, or sweet wormwood (*Artemisia annua*)	Profound cytotoxic activity against tumor cells.[22] Exhibits antiangiogenic and apoptotic activity.[23] Selectively cytotoxic against cancer cells due to their higher iron content than normal cells.[24]	Low toxicity except for reports of selective neuronal toxicity in brainstem; dogs especially sensitive to this adverse effect.[25]	Should not be given in combination with radiation therapy or within 2 months following radiation treatment because radiation therapy causes iron release into surrounding tissue; can be given with chemotherapy.[26] Grapefruit concentrate may increase absorption; high levels of antioxidants may compromise cytotoxic effects.[27]

*The information in this table is intended to describe various botanical compounds that have been used in cancer treatments. Instruction on correct dosages, routes of administration, and guidelines for veterinary practice can be found elsewhere.

TABLE 17-1   Botanical Compounds with Purported Anticancer Activity—cont'd

Name and Description	Reported Effects in Cancer Cells or Model Systems	Adverse Effects and Precautions	Interactions with Other Herbs, Drugs, Foods, or Treatments
Asian ginseng (*Panax ginseng*)	Antitumor effects and cellular immunity stimulation.[28] May hypersensitize multidrug-resistant tumor cells to chemotherapy.[29]	Considered safe at recommended dosages, but may cause overstimulation and insomnia. Caution required for hypertensive individuals.	Caution advised when given concomitantly with warfarin, oral hypoglycemic medications, insulin, and phenelzine.[30] Has been shown to increase antibody levels and improve immune function after vaccination compared with placebo.[31]
Astragalus (*Astragalus membranaceus*)	"Chemotherapy support" (i.e., commonly prescribed by Chinese medical practitioners to reduce the side effects, promote immune function, and increase survival time in patients undergoing chemotherapy and radiation therapy). May enhance secretion of TNF, improve function of T lymphocytes, and stimulate interferon production.[32]	Reports exist that Astragalus can induce hypotension, diuresis, dizziness, and fatigue; also, that overdosage may cause immunosuppression.[33]	May potentiate activity of chemotherapeutic agents, inhibit recurrences, prolong survival time, and reduce adverse toxicities of antineoplastic agents.[34-36]
Bloodroot, or sanguinaria, from (*Sanguinaria canadensis*)	Sanguinarine is effective against multidrug resistance in human cervical cells via bimodal cell death.[37] Exhibits differential antiproliferative and apoptotic responses for cancer cells versus normal cells.[38] Induces growth inhibitory and antiproliferative effects in human prostate carcinoma cells regardless of their androgen status.[39]	If used topically as an escharotic, bloodroot may cause serious scarring.[40]	Sanguinarine and zinc interact synergistically against bacteria.[41]
Bromelain (from pineapple, or *Ananas comosus*)	One of several oral proteolytic enzymes promoted for the relief of symptoms associated with cancer and cancer therapies, thereby improving quality of life.[42] Stimulate immune response, reduces inflammation, and inhibits metastasis.[43]	Usually well tolerated. High dosages may produce gastrointestinal disturbances.	Interacts with aspirin.[44] May increase risk of bleeding or potentiate effects of warfarin.[45]

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
Cat's claw (*Uncaria tomentosa*, or *Uña de gato*)	Historically used by native Amazonians to treat tumors.[33] Antioxidant.[46]	May inhibit platelet aggregation; high tannin content may destroy nutrients or impede absorption.[33] Some manufacturers may substitute 1 of more than 30 other plants that share the same name but not the same benefits, leading to potentially harmful effects.[33]	Works synergistically with medicinal mushroom extracts plus nicotinamide plus zinc to optimize immunostimulatory actions.[47] May inhibit cytochrome P450 CYP3A4.[48]
Chaparral (*Larrea tridentate*)	Unproven anticancer herb.	Causes multiple serious and potentially fatal adverse effects; may elevate liver enzymes, lower serum glucose values, and induce irreversible kidney failure and creatinine elevations.[49] Considered unsafe by the U.S. FDA. Hepatotoxic[50] and likely carcinogenic.[51]	None found.
Comfrey (*Symphytum officinale*)	Unproven anticancer herb.	Contains hepatotoxic pyrrolizidine alkaloids (PAs). Comfrey is mutagenic, carcinogenic, causes hepatic veno-occlusive disease, and may be a cause of pulmonary hypertension.[52,53] Comfrey proponents claim that comfrey tea consumption is safe (saying that PAs are insoluble in water), that small amounts of comfrey are nontoxic[54]; both assumptions are untrue. In fact, the concentration of PAs in comfrey tea may be underestimated substantially, and long-term ingestion can be fatal,[55] as PA toxicity is cumulative.[56] Several countries have banned the sale of herbal products containing comfrey; the FDA advised the industry to remove comfrey from the market in 2001.[57]	Concurrent administration of comfrey with potent microsomal enzyme inducers such as phenobarbital enhances the toxicity of pyrrolizidines, lending support to the idea that the cytochrome P450 system may form toxic compounds from PAs.[53]

Continued

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
Cordyceps sinensis (originally a fungal parasite on *Lepidoptera*/Chinese caterpillar larvae; now cultured) and other *Cordyceps* spp.	Water extracts of *Cordyceps sinensis* (WECS) induced apoptosis *in vitro*[58,59] and showed antimetastatic activity in mice.[60] Antiangiogenic properties.[61]	None found.	None found.
Curcumin *(Curcuma longa)*	Antiinflammatory, antiproliferative effects in cancer cell lines, promotes cell cycle arrest, induces apoptosis; possible antiangiogenic effects; impedes tumor growth and metastasis; provides symptomatic relief for patients with ulcerating oral or cutaneous squamous cell carcinoma who had failed to respond to standard treatment.[62,63] Helps control matrix metalloproteinase activity.[64]	None found.	May selectively enhance the cytotoxicity of chemotherapy and reduce negative side effects; protects against bleomycin- and cyclophosphamide-induced pulmonary fibrosis; reduces chemotherapy-induced inflammation; protects against doxorubicin-induced nephrotoxic effects, may augment effects of cisplatin against fibrosarcoma.[62]
Essiac tea, based on a Native American formula, named after Rene Caisse (Essiac is her last name spelled backward); a related product, Flor-Essence, contains the original four herbs plus watercress, blessed thistle, red clover blossom, and kelp	Possibly antitumor or immunomodulatory. The individual herbs have inconsistent antitumor activity, but tests of the whole product found no tumor-reducing activity.[33]	Toxicity of Essiac tea would be related to that of the individual herbs. Occasional nausea has been reported.[33]	Watercress can interfere with the cytochrome P450 system.[65] Red clover has the potential to interact with coumarin.[66]
Frankincense, boswellic acids, salai guggul *(Boswellia serrata, Boswellia carteri)*	Exhibits potent growth inhibition and cytotoxicity on malignant glioma[67] and leukemia cell lines,[68,69] and on primary cell cultures established from human meningioma specimens.[70] Herbal extract induces apoptosis on leukemic and glioma cell lines.[71]	May cause mild gastrointestinal (GI) side effects (loose stools, nausea, epigastric pain)[73] but otherwise has low incidence of negative side effects.	Plasma levels of *Boswellia serrata* gum resin were only detectable when administered with a high-fat meal versus a fasted state.[74]

TABLE 17-1   Botanical Compounds with Purported Anticancer Activity—cont'd

Name and Description	Reported Effects in Cancer Cells or Model Systems	Adverse Effects and Precautions	Interactions with Other Herbs, Drugs, Foods, or Treatments
Ganoderma lucidum, referred to as *reishi* mushroom in Japanese, or *lingzhi* in Chinese	Reduces inflammation.[62] Induces differentiation and apoptosis in highly metastatic melanoma and fibrosarcoma cells.[72] Chemotherapy support to treat general fatigue and weakness. Tumor inhibition, immune activation, hepatoprotection, histamine inhibition.[75] Methanolic extract of *G. lucidum* provided a significant preventive effect against cisplatin-induced nephrotoxicity.[76]	Rare; after continuous use over several months, the following may appear: dizziness, dry mouth and throat, epistaxis, abdominal upset. May increase bleeding time. A 4-week trial of *G. lucidum* extract revealed no evidence of liver, renal, or DNA toxicity inhealthy human subjects.[77]	Not recommended for those taking anticoagulant medications.
Garlic (*Allium sativum*)	Cancer preventive.[78] Preliminary studies of garlic powder added to dry dog food offered no significant immunomodulatory benefits in normal dogs.[79]	Should be discontinued prior to surgery due to antiplatelet effects. Large dosages may cause gastrointestinal side effects.[80] May cause Heinz-body anemia in small animals.[81]	Caution is advised when used in conjunction with other agents possessing antiplatelet effects (ginger, ginkgo, vitamin E, pharmaceuticals). Garlic extracts inhibit various cytochrome P450 enzymes; may also induce the activity of conjugating enzymes such as glutathione S-transferases and quinone reductases.[82]
Ginger (*Zingiber officinale*)	Antinauseant and antiemetic.[83] Antiinflammatory.[84]	Rare; sensitive individuals may experience GI irritation. Caution advised in individuals with clotting disorders.	Avoid use with other antiplatelet agents, including herbs such as ginger, ginkgo, vitamin E.
Hoxsey therapy—a combination of internal and external preparations with dietary specifications, vitamin and mineral supplementation, along with attitudinal counseling[85]	The exact components of the herbal mixtures are held in secrecy but may contain compounds with antitumor activity.[1] Peer-reviewed scientific literature and case histories submitted by Harry Hoxsey failed to provide acceptable evidence of treatment efficacy.[33]	Some of the herbs in the formula such as pokeweed and buckthorn can have serious adverse effects such as respiratory paralysis and severe diarrhea.[1] The external remedies involve escharotics; the internal formula contains a mixture of about nine herbs, including cascara, which can have abundant laxative effects.[33]	Pokeweed constituents can increase the transport of macromolecules and hydrophilic drugs across the intestinal epithelium.[86] Cascara can decrease the absorption of drugs.[87]

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
Kombucha tea (also known as Manchurian or Kargosok tea)—derived from the fermentation of various yeasts and bacteria	Exhibited potent antioxidant and immunomodulating properties in a study on rats.[88,89] Mice ingesting Kombucha on a chronic basis demonstrated significantly inhibited weight gain, increased longevity and environmental awareness and responsiveness; these mice also exhibited longer spleens and hepatomegaly.[90] Contains antimicrobial compounds.[91]	Home-brewed versions are prone to microbial contamination, as the culture must grow at room temperature for 7 to 10 days, and the acid mixture can leach lead and other toxins from certain containers.[33] May be associated with metabolic acidosis and possibly death.[92] Associated with a case of anti-Jo1 myositis with pleural effusions and pericardial tamponade,[93] liver damage, gastrointestinal toxicity,[94] and cutaneous anthrax infection.[95] The increased energy some people report may be due to the caffeine and large amounts of sugar in the mixture.[96] Documented risks do not outweigh the "largely undetermined" benefits of Kombucha.[95]	None found.
Ligustrum (*Ligustrum lucidum*)	Immune stimulant; immunomodulatory.[97]	None reported with normal dosing. Symptoms of overdose may include vomiting and dizziness.[98]	None found.
Maitake mushroom (*Grifola frondosa*)	Maitake beta-glucan MD-fraction (MDF) acts in a dose-dependent manner directly on hematopoietic bone marrow cells (BMC) and enhances their growth and differentiation into colony forming cells; MDF also protected the granulocyte-macrophage colonies from doxorubicin toxicity *in vitro*.[99]	None found.	None found.
Milk thistle	Silybin, a major flavonoid constituent of milk thistle extract, has been shown to have anticarcinogenic and antiproliferative effects in several in vitro and animal models.[100]	In early clinical trials of the active ingredient (silibinin) in people, diarrhea and elevations in bilirubin have been observed.	Silymarin has shown synergistic effects with cisplatin and doxorubicin *in vitro*, reduction of nephrotoxicity from cisplatin, and lack of interference with chemotherapy in cases of acute promyelocytic leukemia.[31]

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
			Can inhibit some cytochrome P450 isoforms, possibly due to its five hydroxyl groups on the silybin molecule.[55] However, silibinin may offer synergistic anticancer effects when combined with conventional cytotoxic agents.[101]
Mistletoe extract (*Viscum album*, Iscador)[102]	Methodologically stronger trials fail to demonstrate efficacy reported in weaker studies.[103] Extracts of mistletoe kill cancer cells and stimulate immune function.[104,105] Has a beneficial effect on quality of life of cancer patients, possibly related to modulation of the immuno-neuroendocrine axis and β-endorphin release.[106] Beneficially reduces side effects of chemotherapy in cancer patients.[107] Proapoptotic.[108]	May cause drug-induced hepatitis.[109] Some have expressed concerns that since mistletoe can grow as a parasite on toxic host plants, it may thereby absorb harmful alkaloids and glycosides; in addition, self-dosing with the herb is considered excessively dangerous, as numerous poisonings and deaths from mistletoe berries have occurred.[33]	Mistletoe lectin can enhance cytotoxicity of anticancer drugs *in vitro*.[110,111]
Noni juice (from *Morinda citrifolia*, or the Indian mulberry plant)	Certain noni fruit extracts may have antiproliferative activity and inhibit cancer growth.[112] The National Institutes of Health has provided support for a phase I clinical study of freeze dried noni fruit extract in cancer patients.[113] A polysaccharide-rich substance from noni showed synergistic or additive beneficial effects when combined with a broad spectrum of chemotherapeutic drugs, including cisplatin, doxorubicin, vincristine, and others.[114]	A case of hyperkalemia has been reported following use.[115]	Possibly linked to coumadin resistance.[116]

Continued

TABLE 17-1 Botanical Compounds with Purported Anticancer Activity—cont'd

Name and Description	Reported Effects in Cancer Cells or Model Systems	Adverse Effects and Precautions	Interactions with Other Herbs, Drugs, Foods, or Treatments
Pau d'arco (also known as *taheebo*, *lapacho*, and *ipe roxo*)	Pau d'arco contains lapachol, which has antitumor properties.[33]	Human studies in the early 1970s showed that undesirable side effects from lapachol appeared once significant plasma levels occurred, forcing discontinuation of the investigational new drug; even low doses can cause nausea, vomiting, and problems with blood clotting.[33]	None found.
PC-SPES (a combination of eight herbs)	Demonstrates some clinical efficacy for hormone-sensitive and hormone-refractory prostate cancer; phase II clinical trial underway, comparing PC-SPES with diethylstilbestrol for hormone-refractory prostate cancer.[117] Appears to decrease levels of prostate-specific antigen (by over 50%, even in men refractory to conventional hormone-ablative therapy), stabilize or improve metastatic disease, reduce pain, and improve quality of life.[118] May prove useful as a radiation sensitizer.[119]	Adverse effects appear to stem from estrogenic properties of PC-SPES: thrombotic events, breast tenderness or gynecomastia, sexual dysfunction, hot flashes, edema, loss of body hair, and hypertriglyceridemia.[74] Other adverse effects include gastrointestinal symptoms, fatigue, leg cramping, and allergic reactions.[75]	No data on interactions with chemotherapy or radiation are available.[75]
Perillyl alcohol (monoterpene isolated from essential oils of lavendin, peppermint, spearmint, cherries, celery seeds, and other plants)	Induces cell-cycle arrest and apoptosis.[120] Antiangiogenic, with tumor chemopreventive and chemotherapeutic activity.[121,122] Inhibits cell proliferation, suppresses cancer cell growth and metastasis.[123] May stimulate or sensitize cells to apoptosis.[124] A phase II trial of daily perillyl alcohol in patients with advanced ovarian cancer failed to offer significant clinical benefit, defined as freedom from progression of disease after 6 months of therapy.[125]	Most common adverse effects from orally dosed perillyl alcohol were gastrointestinal related (nausea, vomiting, anorexia, unpleasant taste, diarrhea); other effects include fatigue, slurred speech, short-term memory loss, ataxia, and hypokalemia.[78]	Hyperthermia synergistically potentiates the cytotoxicity of perillyl alcohol against cancer cells.[127] Perillyl alcohol enhances the sensitivity of head and neck cancer cells to radiation treatment *in vitro*.[76]

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
	A phase II multicenter trial of perillyl alcohol inpatients with advanced hormone refractory prostate cancer showed lack of objective benefit and poor drug tolerability.[126]		
Pycnogenol (trade name for a standardized substance extracted from the French maritime pine tree)	*In vitro* and animal studies suggest that pycnogenol can prevent oxidative damage and improve natural killer cell cytotoxicity.[33]	Occasional allergic reaction or diarrhea if start on a high dosage.[33]	None found.
Schisandra (*Schisandra chinensis*)	Apoptosis inducer.[128] Hepatoprotective.[129] Acts as a weak phytoestrogen.[130]	Uncommon; include gastrointestinal upset, anorexia, and dermatitis.	Gomisin C, a component of Schisandra fruit, is a mechanism-based inhibitor that competitively inhibits and irreversibly inactivates cytochrome activity, specifically CYP3A4.[131]
Scutellaria barbatae	Cytotoxic to 100% of actively proliferating ovarian cancer cell lines tested and 50% (two of four) actively proliferating breast cancer cell lines.[132] Induces apoptosis.[132,133] Inhibits intracellular aromatase in myometrial and leiomyomal cells.[134]	None found.	None found.
Shiitake mushroom (*Lentinan edodes*)	Antitumor,[135] immune-regulating, antiviral, antimicrobial, antiparasitic, hepatoprotective, and cancer preventive.[29] Stimulates the production of T lymphocytes, macrophages, and natural killer cells.[33]	High dosages may cause diarrhea and bloating.	A combination of lentinan and didanosine in HIV-positive patients improved CD4 cell counts significantly longer than didanosine alone.[136]
Siberian ginseng (*Eleutherococcus senticosus*)	Cytotoxic and antioxidant.[137] Improves quality of life.[138]	None found.	May alter digoxin levels.[139,140]

TABLE 17-1	Botanical Compounds with Purported Anticancer Activity—cont'd		
Name and Description	**Reported Effects in Cancer Cells or Model Systems**	**Adverse Effects and Precautions**	**Interactions with Other Herbs, Drugs, Foods, or Treatments**
Tea, green or black	Stimulates production of immune cells; may decrease cancer risk.[141-143] Inhibition of photocarcinogenesis in mice is associated with angiogenic factor inhibition and antitumor immune reactivity induction.[144] Suppresses dioxin-induced transformation of the aryl hydrocarbon receptor.[145] Black tea appears to soothe the discomfort associated with radiation-induced oral mucositis due to tannic acid and other ingredients.[146]	Epigallocatechin-3-gallate, from green tea, negatively affects reproductive performance in swine.[147]	May interfere with orally administered codeine, theophylline, and atropine due to the high amount of tannins; stimulant effects due to caffeine may be additive with other sympathomimetics.[31] Tea polyphenols may interact with cytochrome P450 enzymes.[148] Green tea extract induces a nearly doubled increase in hepatic CYP1A2 levels and therefore may reduce the rate and amount of absorption of other drugs.[149]
Ten significant tonic decoction (*Shi Quan Da Bu Tang*)[150]; also known as *Juzen-taiho-to*, or *TJ-48*; a Japanese herbal (Kampo) medicine	Stimulates immune function, hematopoiesis, inhibits tumor growth, protects against renal and hepatic toxicity and bone marrow suppression caused by cisplatin. Reduces leukopenia, thrombocytopenia, weight loss associated with mitomycin.[151] Biological response modifier, reducing side effects from chemotherapy and radiation, and improving general condition of patients.[152]	Adverse effects are rare for Kampo herbs in general when they are properly prescribed, but acute interstitial pneumonitis and hypokalemia have occasionally been reported.[153]	None found.

Table 17-2 lists several nutraceuticals used in the treatment of cancer. (A full examination of nutrition and cancer is available in Chapter 16, Section B.) Many questions persist, especially concerning those with strong antioxidant activity: Do they interact with chemotherapeutic agents? Do some chemotherapy drugs require a pro-oxidative environment? When is best to include antioxidants? Or should antioxidant therapy be discontinued during chemotherapy?[105] Especially perplexing and vexing is the finding from animal studies that a single antioxidant may either potentiate or inhibit radiation efficacy, depending on the dosage and timing of antioxidant administration relative to irradiation. Dual effects also occur with antioxidants given with chemotherapy. As such, Weiger et al. have advised: "[I]t seems prudent to discourage concurrent use of nonprescription antioxidants with radiation therapy or chemotherapy until more evidence becomes available."[74]

Acupuncture

Acupuncture involves the insertion of thin, sterile needles into precise anatomic locations that yield reproducible effects through neuromodulation. Side effects are rare.[165]

TABLE 17-2   Nutraceuticals Used in the Treatment of Cancer

Nutraceutical or Nutritional Component	Potential Advantages	Potential Adverse Effects or Other Drawbacks
Coenzyme Q10[155]	Chemotherapy support; antioxidant; immune stimulant.[156] Aids metabolic reactions. May protect against doxorubicin-induced heart damage.[157]	May reduce response to radiotherapy if administered concurrently; patients may experience headache, fatigue, and heartburn with large doses; treatment may interact with warfarin.[158]
Fish oils (eicosapentanoic acid [EPA] and docosahexanoic acid [DHA])	Inhibit cancer cell proliferation, invasion, and progression; prompt cell cycle arrest or apoptosis, induce cancer cell differentiation; possible antiangiogenic properties. May increase the cytotoxic efficacy of chemotherapy by increasing drug delivery across tumor cell membranes; may increase the therapeutic efficacy of radiation.[62]	Dose-limiting side effects are usually gastrointestinal (diarrhea, esophageal or gastric irritation) that diminishes when fish oils ingested with meals. Must be taken with augmented vitamin E to protect against elevated lipid peroxidation and depletion of antioxidants, particularly tocopherols.[62]
Genistein (from soy)	Cancer preventive, though studies are mixed.[159]	Phytoestrogenic; may stimulate estrogen receptors in certain tumors; may affect the response of breast cancer to tamoxifen.[160] Genistein inhibits cytochrome P450 enzymes and interacts with transporters P-glycoprotein, MRP1, and MRP2.[38]
Glutathione	Reduces neurologic toxicity of cisplatin, improves quality of life.[161,162]	Research supports use of intravenous glutathione over oral administration.
Quercetin	Downregulates mutant p53 protein, arrests cell cycle at G_1, inhibits tyrosine kinase, inhibits heat shock proteins, and suppresses *ras* protein expression; demonstrates antitumor effects against a wide range of cancers; potentiates the therapeutic efficacy of cisplatin, adriamycin, busulphan, and cyclophosphamide; decreases resistance to gemcitabine and topotecan.[62]	Usually well tolerated.
Vitamin C	Antioxidant.	Megadose vitamin C shows no advantage over placebo therapy in terms of disease progression or objective improvement in measurable disease; adverse effects include diarrhea, renal stones, iron overload, and gastrointestinal discomfort.[163]
Vitamin E	Antioxidant. May reduce severity of mucositis.[164]	Hypervitaminosis E is uncommon.

Acupuncture is becoming increasingly prevalent in cancer clinics, likely because of its effectiveness and evidential support. In 2002, 86% of respondents queried at the Naval Medical Center's Oncology Clinic in San Diego considered it to be very important that the clinic continue to provide acupuncture services, and most patients showed at least some improvement in their symptoms following treatment.[31] As such, acupuncture can play an important role on several levels in the care and support of cancer patients, as shown in Table 17-3.

TABLE 17-3 Cancer-Related Ailments That May Benefit from Acupuncture	
Cancer-related Ailment	**Evidence of Effects of Acupuncture**
Toxic side effects of radiation and chemotherapy	Reduces leukopenia, gastrointestinal, and systemic reactions to increase patient participation in conventional treatment.[166]
Circulatory improvement	Reduces edema in the extremities.[151]
Cancer-related breathlessness*[167]	Marked symptomatic benefit, with significant improvements in breathlessness, relaxation, and anxiety, reported in 70% of patients.[168]
Postoperative nausea and nausea induced by chemotherapy or opioid therapy	Acupuncture is often effective in reducing emetic effects of chemotherapy and opioids.[169-173] Acupressure bands show less effective benefits following cardiac surgery for reduction of postoperative nausea and vomiting,[174] although digital acupressure was successful in reducing nausea, vomiting, and dry retches during early pregnancy.[175]
Postchemotherapy fatigue	Patients experienced a mean improvement of 31% in a single-arm, phase II, pilot study.[176]
Immune hypofunction	Natural killer cell cytotoxicity enhanced.[177] Increases the percentage of peripheral blood T lymphocytes.[178] Enhances production of circulating interferon, interleukin-2.[179,180]
Malignant pain, including: postradiation fibrosis, muscle spasm, vascular problems, hyperesthesia, dysaesthesia, and resistant bone pain	A combination of herbal drugs and electro-acupuncture markedly reduced the amount of analgesic and sedative medication needed by 212 patients with bone metastasis.[181] Auricular stud acupuncture provided pain relief in terminally ill patients with neuropathic, soft tissue, bone, and malignant pain, in whom pain remained uncontrolled by conventional means.[182-184] Controls intractable and malignant pain.[185-189]
Phantom limb pain (PLP) of amputees	Marked improvement in 62% of patients and moderate improvement in 23%.[190]
Poorly healing wounds	Acupuncture treatment with electrical stimulation and laser facilitated wound healing of horses with granulomatous, ulcerating, poorly healing wounds.[191]
Postoperative ileus	Acupuncture group had restoration of GI motility in less than half the time required by the control group.[192]
Postoperative pain	Acupuncture, in combination with Chinese herbs and epidural morphine, relieved pain in postoperative patients with liver cancer, and reduced untoward side effects from morphine.[193]
Preoperative anxiety	Human patients receiving acupuncture experienced less palmar sweating prior to anesthesia.[194]
Radiation-induced xerostomia	Sixty-eight percent of patients receiving classical acupuncture and 50% of patients receiving superficial acupuncture had increased salivary flow rates.[195] Acupuncture stimulation significantly increased the release of calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and neuropeptide Y (NPY) in patients with xerostomia.[196] A phase I-II study on the use of acupuncture-like transcutaneous nerve stimulation for the treatment of radiation-induced xerostomia showed that this form of treatment improved whole saliva production and related xerotomia symptoms and that the treatment effects lasted at least 6 months.[197]

*Up to 70% of humans with a wide range of malignancies experience dyspnea in the last 6 weeks of life. Dyspnea, or breathlessness, may be caused by the tumor itself, the treatment of the tumor, coexisting cardiopulmonary disease, or a combination of these ailments. The pathophysiology of breathlessness in cancer is poorly understood and probably involves diverse factors ranging from chemical stimulation of the medullary respiratory center to the tone of the muscles of respiration. Although it is a common and distressing symptom in human patients with advanced cancer, it is extremely difficult to palliate once specific disease-oriented measures are exhausted.[46,47]

Massage and Manipulative Therapy

Cancer and its treatments can make people and animals physically uncomfortable. In addition to pain relief, gentle manual therapy has much to offer patients suffering from cancer and its treatment, such as improved immune, circulatory, and visceral functions. Veterinary massage, while lacking in research, is growing in popularity.[198,199] The list that follows includes reports of benefits of soft tissue manipulative medicine and massage in humans; home-care providers can be instructed in several of these procedures to assist in patient recovery and providing of comfort.

1. Pain and stress relief[33,200-206]
2. Immune enhancement[98]
3. Alleviation of lymphedema[74,207-210]
4. Relief of anxiety, nausea, depression, and fatigue[211]

One should avoid forceful maneuvers in cancer care in which excessive force applied to sites such as bony metastasis could lead to deleterious results. And, while no evidence exists indicating that massage promotes the likelihood of tumor metastasis, one should avoid massage directly over known tumors or predictable metastasis sites.[74] Tissues subjected to prior surgery or radiation therapy may be fragile, and as such massage to these areas should either be avoided or done gently. Hypercoagulable patients may experience emboli subsequent to deep pressure over a thrombus; patients who are prone to bleeding may develop hematomata secondary to pressures that, in normal patients, would not cause problems. Deep abdominal massage has caused internal bleeding even in the absence of bleeding disorders.[173] Massage to sites containing stents or prostheses may cause displacement.

Homeopathy

Homeopathy is a CAM approach in which patients receive minute dilutions of natural substances (from plants, minerals, or animals) in order to stimulate that individual's natural healing response. According to homeopathic principles, a natural substance that causes symptoms of illness in a healthy person can cure that same sickness in an unhealthy person suffering from those same symptoms, when it is administered in a highly dilute form. To select a remedy for a patient, a homeopath first determines which substance most closely resembles the symptoms of that patient's disease and then selects a specific remedy to match the clinical picture.

Contrary to conventional medical pharmacotherapeutic principles, homeopathic doctrine states that remedies are more powerful as they become more dilute. A matter of consternation among those accustomed to conventional medical approaches is the fact that highly diluted remedies may not even contain one molecule of the original substance.[212] The mechanism of purported action of homeopathy remains unknown.

Proponents speculate that homeopathic remedies may have properties distinct from those of water, although skeptics would argue that there is little, if any, difference between homeopathic remedies and water.

No single remedy exists for treating cancer with homeopathy, and homeopathy is not considered a self-care option for cancer patients.[85,213] While homeopaths may administer remedies to serve as a complementary treatment to minimize side effects of conventional treatment methods, they have not as often attempted to treat the cancer itself.[62] Homeopathy in general is unlikely to harm patients unless it is used as a substitute for proper conventional care.[214]

Evidence for the incorporation of homeopathy for symptom control in cancer patients is growing, but critics remain unconvinced, mainly because of the widespread methodological flaws in most studies.[213] Reports from human studies support the use of homeopathy for symptoms of pain, fatigue, hot flushes, and mood disturbances[215,216]; control of skin reactions during radiotherapy for breast cancer[217,218]; chemotherapy-induced stomatitis in children[219]; and hot flushes for women receiving tamoxifen.[220] One study investigating the effects of a homeopathic drug, Chelidonium, reported antitumor and nongenotoxic activity, as well as favorable modulation of certain marker enzymes.[221,222] Another study indicated that a homeopathic medication called TRAUMEEL S significantly reduced the severity and duration of chemotherapy-induced stomatitis in children undergoing bone marrow transplantation.[219]

Reactions to homeopathic remedies are reportedly common but usually minor and are referred to as "aggravations."[223]

Aromatherapy

Few studies on the effects of aromatherapy on cancer patients are available.[213,224] Those that do exist speak mainly about the psychological benefits (e.g., anxiety reduction, quality of life improvement); others combine aromatherapy with massage, making it unclear whether the aromatherapy per se offered any unique benefits.[225,226] Extrapolating the evidence from human studies to nonhumans will be difficult because of the technical challenges to objectively quantify and document pleasures, sensory changes, or aversions resulting from inhaling aromatic compounds across species. Further, the scents that dogs and cats enjoy often seem to vary widely from those humans find attractive.

Miscellaneous Treatments

Table 17-4 lists some additional compounds that have been used in cancer treatment. Several have been shrouded in controversy and may not be available in the United States and other countries.

TABLE 17-4   Miscellaneous Treatments and Controversial Compounds

Treatment or Compound	Purported Indications and Mechanisms	Adverse Effects	Controversy
714-X (A formula made of camphor, ammonium chloride, ammonium nitrate, sodium chloride, ethyl alcohol, and water)	Purported to improve immune function by inhibiting cancer cells through the release of camphor and nitrogen	The Canadian Expert Advisory committee has warned of possible adverse effects from the combination of 714-X constituents.[227]	No formal clinical, animal, or cell-system studies documented safety or efficacy. The U.S. Food and Drug Administration (FDA) has imposed an import ban on 714-X.[228]
Coley's toxins; Endogenous tumor necrosis factor (TNF) therapy from *Streptococcus pyogenes* products	Immunostimulating; increases production of natural TNF by the reticuloendothelial system, with less toxic effects than recombinant TNF.[229]	Fever-related symptoms of less severity than recombinant TNF; may cause metabolic acidosis.[151]	Clinical studies assessing efficacy alone or in combination with conventional cancer therapy are limited.[230]
Energy work— various touch or nontouch therapies (including Reiki and healing touch) that supposedly clear patients' auras, remove "energy blockages," or send "healing energy" to the patient; certain nontouch therapies are practiced without the patient in the immediate vicinity of the practitioner	Studies in human cancer patients with both significant and nonsignificant findings show that healing touch recipients often report improvements in mood and feelings of well-being; reduction in pain, blood pressure, and fatigue; improved vitality, and satisfaction with treatment.[231] A phase II trial of Reiki in patients with advanced cancer found that Reiki helped improve pain control and quality of life.[232]	None.	"Energy" therapists who explain that they work with a patient's auric field and spirit guides may offend certain clients' religious belief systems.
Escharotic or caustic pastes, used for skin cancer to destroy lesions (sold as products called Cansema, Curaderm, and HerbVeil8)	Zinc chloride and *Sanguinaria canadensis* (bloodroot) are used as part of the Mohs chemosurgery fixed-tissue technique, but using escharotics without surgery has been discredited by allopathic practitioners, yet it persists as an alternative cancer treatment.[40]	Case reports of patients opting to forego conventional and potentially curative skin cancer treatment, in favor of topical treatment with *Sanguinaria canadensis*, have faced scarring and recurrence.[227]	"[A]lternative therapy for cutaneous malignancy can lead to a false sense of security and may result in substantial morbidity if not mortality."[227]
Glandular therapies	Consuming glands or tissues of "healthy" animals supposedly benefits those same substances in an ill animal.	Theoretical risk of bovine spongiform encephalopathy (BSE), especially from brain and other central nervous system extracts.	FDA banned high-risk bovine materials from older cows in foods and supplements in 2004 but still allows high-risk

TABLE 17-4	Miscellaneous Treatments and Controversial Compounds—cont'd		
Treatment or Compound	**Purported Indications and Mechanisms**	**Adverse Effects**	**Controversy**
	In vitro experiments of thymus extracts indicate that they activate natural killer cells and increase cytotoxic activity. Studies in rats and mice show that thymus extracts may inhibit tumor growth. However, in a systematic review of 13 studies on thymus therapy, 5 suggested there may be some benefit, but overall, the results were not positive, and studies were largely of low methodological quality.[192]	Injectable thymus preparations can cause severe allergic reactions.[192]	parts from cows under 30 months.[57]
Cure for All Cancers (Hulda Clark)[233]	Based on Hulda Clark's claim that parasites cause cancer, the Clark organization sells a Super-Zapper Deluxe. This is a low-voltage electrical device that supposedly cures all forms of cancer, especially when used with the Complete Herbal Parasite Program.[234]	Although the Zapper supposedly does not harm human tissue, the Zapper has caused pacemaker malfunction and severe tissue burn.[230]	Clark and her associates have repeatedly faced legal challenges. One case in 1999 was filed by a breast cancer patient. Court papers stated that she was told that dust in her apartment caused her breast cancer. She would develop leukemia if she returned to her apartment because of her blood type. She had a "rabbit fluke" inside her breast, and many other bizarre claims.[230]
Hydrazine sulfate	Had been advocated for cancer cachexia.[235] Had been thought to result in significantly greater caloric intake, albumin maintenance, and prolong survival times.[236] Was thought to have antitumor effects.[237] However, three prospective, randomized, placebo-controlled trials in the United States found no benefit.[192]	Significant adverse neurologic effects and possible liver damage has been reported, but relatively infrequent.	Three prospective trials have shown no benefit when hydrazine sulfate was added to standard treatments.[120] Hydrazine sulfate has been shown to increase the incidence of lung, liver, and mammary tumors in laboratory animals.[238]

Continued

TABLE 17-4	Miscellaneous Treatments and Controversial Compounds—cont'd		
Treatment or Compound	**Purported Indications and Mechanisms**	**Adverse Effects**	**Controversy**
Oxygenation therapies (hydrogen peroxide, ozone)	Oxygenating the blood supposedly can "exert a negative effect on the cancer cell" with the idea that cancer cells are anaerobic and that substances such as ozone can harm cancer cells as much as chemotherapy but without damage to normal cells.[239] This notion rests on the incorrect assumption that cancer arises as a result of oxygen deficiency and that overwhelming cancer cells with more oxygen than they can manage will be cytotoxic.[192]	Airborne ozone from ozone generators can irritate mucous membranes of nose and throat at 0.01 ppm; concentrations of 1 to 10 ppm can cause headaches, respiratory irritation, and possibly coma.	No evidence that ozone generators are safe and effective cancer treatments. The practice of *ex vivo* ozone therapy involves exposing up to 300 ml of freshly drawn blood to a gas mixture of oxygen and ozone, then reinfusing this mixture back into the patient. Serious complications include hepatitis and death.[240]
Shark and bovine cartilage[241]	Inhibits angiogenesis.[242] Augments cellular immune response and T-cell infiltration into tumors.[243] Cytotoxic.[244] May limit spread of bone metastasis in rodents.[245] A phase III trial is currently ongoing in the United States, Canada, and Europe using Neovastat (AE-941), a purified, shark-derived antiangiogenic factor.[246]	May contribute to the onset or severity of hypercalcemia.[247] Pregnant or young/growing individuals should not take shark cartilage since the antiangiogenic effects could be harmful.[120] Nausea, vomiting, constipation.[73] No interactions with chemotherapy or radiation therapy reported.[74]	Ethical consequences of diminishing shark population since large dosages are required.[151] As noted by Ostrander et al., in response to the incorrect but popular argument that sharks rarely or never get cancer: "Sharks do get cancer." "The rate of shark cancer is not known from the present data." "Even if the incidence of shark cancer were low, cancer incidence is irrelevant to the use of crude extracts for cancer treatment."[248]

Signs of a Hoax

Oncology clients sometimes so desperately seek cures for their animals that they may fall victim to ploys of unscrupulous individuals that peddle their "cures" to the unwary. Metz[163] provides the following 10 signs of a hoax to help human patients and their health care providers with detection of fraudulent treatments:
- The treatment is a "secret" that only specific individuals can provide.
- Patients are told not to use conventional medical treatment.
- The treatment promises a cure for almost all cancers or medical conditions.
- The treatment is promoted only in the mass media such as the Internet, television, or radio talk shows and books instead of reputable scientific journals.
- The promoters claim that they are persecuted by the medical establishment.
- Advertisements for the treatment claim to "cleanse the body of poisons and toxins."
- The treatment will help the patient by "strengthening the immune system."

- Testimonials and case reports are used to promote a specific treatment or product.
- Catch phrases such as "nontoxic," "no side effects," and "painless" are used.
- The promoters attack the medical community.[120]

The claims of curing cancer made by Hulda Clark in her book *The Cure for All Cancers* illustrate many of the red flags noted in this list.[233]

GENERAL CONSIDERATIONS WHEN RECOMMENDING INTEGRATIVE CARE FOR ONCOLOGY PATIENTS

While CAM practitioners are not subject to litigation as often as physicians, referrals to CAM practitioners are not without risk.[249] A referral to a CAM practitioner that delays, prevents, or minimizes the opportunity for the patient to receive necessary care via conventional means, and which subsequently causes the patient suffering, may be considered negligent, as can referrals made to incompetent practitioners.

According to the January 1999 issue of the *Journal of the American Veterinary Medical Association*, "More inquiries and complaints are directed to the American Veterinary Medical Association (AVMA) about alternative and complementary therapies than any other issue."[250] Referring animals for unconventional care can raise issues of CAM provider credibility, especially if the practitioner is not a licensed doctor of veterinary medicine (DVM). To quote the AVMA committee studying this matter:

> [T]he veterinary profession wants to assure the public that, when alternative and complementary modalities are being used, they are being used by persons rendered capable and responsible through education and regulation, and that, as a result, these practitioners will "do no harm." Licensed professionals who have not received adequate education or who do not submit themselves to a required standard of practice in these modalities, and unlicensed nonprofessionals who possess minimal acquired or practical experience, both pose a danger to clients and patients. Furthermore, the committee encourages the use of these modalities within the context of a valid veterinarian/client/patient relationship.[251]

Medical and veterinary schools have an ethical responsibility to their students to provide information about therapies that may yield potential benefits to future patients, which many CAM treatments do. Courses on these topics are increasing in veterinary schools, and a survey showed that 64% of U.S. medical schools offered one or more courses in CAM.[252,253] These schools also need to educate students about ways in which CAM therapies may alter a clinical situation and subsequent diagnosis, and alert future professionals about potential herb-drug interactions.

As more academic institutions provide courses and training programs in CAM techniques, a greater emphasis on scientific exploration will arise, along with substantially more opportunities for methodical and in-depth study. For the treatment of cancer in particular, evidence-based clinical research on the safety and efficacy of CAM modalities in veterinary cancer patients must take place in order to determine where these approaches best partner and synergize with conventional veterinary cancer care. Indeed, as concluded by the American Veterinary Medical Law Association in 2004, veterinarians offering complementary or alternative therapies: "[S]hould remain vigilant to use only what has been demonstrated to be scientifically reliable treatments or therapies. In other words, treatments and therapies that have been subjected to the same critical analysis and assessment as conventional treatments and therapies."[254]

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18

Tumors of the Skin and Subcutaneous Tissues

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INCIDENCE

Tumors of the skin and subcutaneous tissues are the most common tumors affecting dogs, accounting for approximately one third of all tumors encountered in the species.[1-4] In the cat, skin and subcutaneous tumors, which occur second in frequency only to tumors of the lymphoid system, account for approximately one fourth of all tumors in the species.[1,3,5,6] Estimates of annual incidence rates for skin and subcutaneous tumors have been reported to be approximately 450 per 100,000 dogs and 120 per 100,000 cats.[1,7] A large survey calculated the annual incidence rates for nonmelanoma skin tumors as 90.4 per 100,000 for dogs and 34.7 per 100,000 for cats.[8]

Many types of tumors occur on the skin. Table 18-1 presents a list of the 10 most common nonlymphoid cutaneous tumors in the dog based on more than 6000 cases from four continents; Table 18-2 lists the five most common nonlymphoid cutaneous tumors in the cat based on more than 1000 cases. Approximately 75% of the cutaneous tumors encountered in the dog and cat are represented in these two tables. (Mast cell tumors, the most common cutaneous tumor in the dog and the second most common cutaneous tumor in the cat, are discussed at length in Chapter 19.) Approximately 20% to 40% of primary tumors of the skin and subcutaneous tissues are histologically malignant in the dog, compared to 50% to 65% in the cat.[1,3,5-11] One report calculated the odds ratio of the development of malignant cutaneous tumors in dogs based on age and breed.[10] These researchers found that the risk increased linearly by a factor of 1.1 per year of increasing age and that purebred dogs were twice as likely to develop a malignancy as crossbred dogs. Occasionally, cutaneous tumors in dogs and cats occur secondary to metastatic lesions, and the clinician should include this possibility in the list of differential diagnoses. This is particularly important in the cat, in which the syndrome of digital and cutaneous metastasis from pulmonary sites is well documented.

In general, cutaneous tumors occur in older animals. No significant difference in incidence by gender is noted when all types are considered together. Such differences, where they exist, along with breed predilections, are discussed later in the chapter under specific tumor types.

ETIOLOGY

Specific etiologies have been proven for only a few tumors in the dog and cat. Although the etiology of cutaneous tumors likely is multifactorial and largely unknown, current investigations are shedding some light on the subject. Several contributing factors in the development of skin tumors include physical factors (e.g., radiation and thermal injury), genetic and molecular influences, hormones, vaccines, viruses, and immunologic influences.[5,12-27]

Physical Factors

Long-term exposure to the ionizing effects of sunlight results in a solar dermatosis, leading to documented increases in cutaneous hemangioma, hemangiosarcoma, and squamous cell carcinoma in dogs and squamous cell carcinoma in cats.[16-19]

Viral Factors

Several associations have been made between cutaneous tumors and viral etiologies. Papillomavirus has been implicated in squamous papillomatosis (warts) in young dogs, rare instances of papillomatosis of aged animals, pigmented cutaneous papillomatosis (canine pigmented epidermal nevus), and some canine cutaneous squamous cell carcinomas.[13-15,22,25-36]

TABLE 18-1	Frequency (Percentage) of Top 10 Nonlymphoid Cutaneous Neoplasms in the Dog (N = 6,282)
Neoplasm	**Percentage of Cases**
Mast cell tumor	18.8
Hepatoid (perianal sebaceous) adenoma/carcinoma	10.1
Lipoma	7.1
Sebaceous hyperplasia/adenoma	7.1
Histiocytoma	6.7
Squamous cell carcinoma	6.2
Melanoma	6.2
Fibrosarcoma	6.1
Basal cell tumor	4.6
Hemangiopericytoma*	4.4

From Bostock DE: Neoplasms of the skin and subcutaneous tissues in dogs and cats, Br Vet J 142:1-19, 1986; Rothwell TLW, Howlett CR, Middleton DJ et al: Skin neoplasms of dogs in Sydney, Aust Vet J 64:161-164, 1987; Brodey RS: Canine and feline neoplasia, Adv Vet Sci Comp Med 14:309-354, 1970; Finnie JW, Bostock DE: Skin neoplasia in dogs, Aust Vet J 55:602-604, 1979; Mukaratirwa S, Chipunza J, Chitanga S et al: Canine cutaneous neoplasms: prevalence and influence of age, sex, and site on the presence and potential malignancy of cutaneous neoplasms in Zimbabwe, J S Afr Vet Assoc 76:59-62, 2005; and Kaldrymidou H, Leontides L, Koutinas AF et al: Prevalence, distribution and factors associated with the presence and potential for malignancy of cutaneous neoplasms in 174 dogs admitted to a clinic in northern Greece, J Vet Med A Physiol Pathol Clin Med 49:87-91, 2002.

*Many now refer to these lesions as nerve sheath tumors.

TABLE 18-2	Frequency (Percentage) of Top Five Cutaneous Neoplasms in the Cat (N = 1,155)
Neoplasm	**Percentage of Cases**
Basal cell tumor	19.7
Mast cell tumor	17.4
Fibrosarcoma	17.4
Squamous cell carcinoma	11.4*
Sebaceous hyperplasia/adenoma	3.1

From Bostock DE: Neoplasms of the skin and subcutaneous tissues in dogs and cats, Br Vet J 142:1-19, 1986; Brodey RS: Canine and feline neoplasia, Adv Vet Sci Comp Med 14:309-354, 1970; Carpenter JL, Andrews LK, Holzworth J: Tumors and tumor-like lesions. In Holzworth J, editor: Diseases of the cat: medicine and surgery, Philadelphia, 1987, WB Saunders; and Miller MA, Nelson SL, Turk JR et al: Cutaneous neoplasia in 340 cats, Vet Pathol 28:389-395, 1991.

*May be misleading, because one survey[5] did not include ear tumors, a common site for squamous cell carcinoma.

Similarly, papillomaviruses have been implicated in the etiology of papillomas, fibropapillomas (sarcoids), and squamous cell carcinoma in cats.[37-39] The authors have observed several Doberman pinschers with oral papillomatosis that were confirmed to have IgA deficiencies and likely were immunosuppressed, which perhaps contributed to the development of virus-induced papillomas. A report of cutaneous papillomatosis and one of squamous cell carcinoma developing in dogs undergoing immunosuppressive chemotherapy supports this notion.[32,35] The feline leukemia virus (FeLV) is associated with the development of cutaneous lymphoma (see Chapter 31, section B), and under experimental conditions the feline sarcoma virus has produced malignant melanoma in cats.[5] Injection site epithelial tumors have been associated with vaccines produced from active canine oral papillomavirus.[22]

Genetic and Molecular Factors

Recent advances in molecular cytogenetics (see Chapter 1, section A), including gene microarray techniques, have been applied to investigations of chromosomal aberrations in dogs and cats with malignances. Several breed dispositions, such as a disposition for cutaneous histiocytoma in flat-coated retrievers[40] and for nodular dermatofibrosis in German shepherd dogs,[41-48] support the influence of genetic underpinnings. For example, a mutation in the Birt-Hogg-Dubé (BHD) gene has been documented in the disposition for nodular dermatofibrosis in German shepherd dogs.[47,48] A reciprocal translocation also has been documented in a dog with basal cell carcinoma.[49]

As our knowledge of molecular events and tumorigenesis has expanded, several molecular aberrations have been implicated in various canine and feline tumor types. Altered oncogene/tumor suppressor gene expression, epigenetic changes, signal transduction, telomere biology, and death-pathway alterations are common in human malignancies (see Chapters 1 and 2 and Chapter 14, sections B and D), and some that are associated with canine and feline cutaneous tumors have been identified (Table 18-3).[25,27,47,48,50-69] Several molecular events and alterations have been implicated in the genesis of vaccine-associated sarcomas of cats (see Chapter 20). As more is discovered about these important molecular

TABLE 18-3   Molecular and Genetic Factors Associated with Cutaneous Tumors in Dogs and Cats

Factor	Comments	References
Birt-Hogg-Dubé (BHD) gene	This gene is mutated in German shepherd dogs with nodular dermatofibrosis.	47, 48
Transforming growth factor beta-1 (TGF-beta$_1$)	Overexpressed in a cutaneous lesion of German shepherd dogs with nodular dermatofibrosis.	52
p53 gene	Altered expression of this gene, which is crucial for DNA integrity, has been reported in several tumor types in dogs and cats; p53 mutations have been documented in feline cutaneous tumors.	25, 27, 54, 57, 66-68
Metallothionein expression	Can disrupt p53 and has been implicated in canine and feline melanocytic tumors.	55
RB-1 gene	A cell cycle regulatory gene that has been implicated in melanocytic tumors.	57
Cell survival and proliferation factors	Bax/Bcl-2 expression has been implicated in feline basal cell tumors. Relative apoptosis and proliferation rates are altered in several cutaneous tumors.	53, 62-64, 172, 173
Cyclin kinase inhibitors	p21/*waf-1*, p27/*kip1*, and p16/*ink-4a* have been implicated in canine melanocytic tumors.	56, 57, 66, 169
PTEN gene	Altered expression of this important tumor suppressor gene has been implicated in canine melanoma.	57
N-*ras*	Mutation in this protooncogene has been implicated in canine melanoma.	58
Angiogenic factors	Angiogenic factors (VEGF) have been implicated and phenotypic characteristics (integrin expression and vascular density) have been documented in canine squamous cell carcinoma and melanoma.	59-61
Telomere biology	Alterations in expression of telomerase have been noted in many cutaneous tumors.	50, 51
Heat shock proteins	These proteins have been implicated in the control of growth and the differentiation of several neoplasms in humans and have been reported to be overexpressed in canine cutaneous epithelial tumors.	65

and genetic events, several of them may become targets for new therapeutic strategies.

PATHOLOGIC CLASSIFICATION

The heterogeneity of cutaneous structures that may be involved in a neoplastic process complicates the issue of classification. In general, skin tumors are classified histologically according to the tissue of origin (epithelial, mesenchymal, melanotic, or round cell) and the individual cell of origin if sufficient differentiation is present. Tumors are further classified as to the degree of malignancy based on several histologic characteristics. In some cases, no clear differentiation exists between malignant and benign skin tumors.

Clinically, skin tumors are further classified according to the tumor-node-metastasis (TNM) system (Table 18-4)

devised by the World Health Organization (WHO).[70] This system allows the tumor to be described in detail with regard to its clinical presentation. In addition, when a skin tumor is classified, the tumor's location must be considered. Some tumors behave differently in different areas of the body; for example, canine oral melanoma usually is malignant, whereas canine cutaneous melanoma arising from haired skin usually is benign. Also, given the same type of tumor, the biologic behavior in the dog often is different from that in the cat.

HISTORY AND CLINICAL SIGNS

The history for an animal with a cutaneous tumor varies. Often the owner discovers a growth while examining or grooming the pet. Benign tumors are more likely to have a history of slow growth over weeks to years. Pets often

TABLE 18-4	Clinical Stages (TNM) of Canine or Feline Tumors of Epidermal or Dermal Origin (Excluding Lymphoma and Mastocytoma)			
T:	**Primary tumor**			
T_{is}	Preinvasive carcinoma (carcinoma in situ)			
T_o	No evidence of tumor			
T_1	Tumor <2 cm maximum diameter, superficial, or exophytic			
T_2	Tumor 2–5 cm maximum diameter, or with minimal invasion irrespective of size			
T_3	Tumor >5 cm maximum diameter, or with invasion of the subcutis, irrespective of size			
T_4	Tumor invading other structures such as fascia muscle, bone, or cartilage			

Tumors occurring simultaneously should have the actual number recorded. The tumor with the highest T category is selected and the number of tumors indicated in parenthese, e.g., T_2 (5). Successive tumors should be classified independently.

N:	**Regional lymph nodes (RLN)**	**M:**	**Distant metastasis**
N_o	No evidence of RLN involvement	M_o	No evidence of distant metastasis
N_1	Movable ipsilateral nodes	M_1	Distant metastasis detected—specify site(s)
	N_{la} Nodes not considered to contain growth		
	N_{lb} Nodes considered to contain growth		
N_2	Movable contralateral or bilateral nodes		
	N_{2a} Nodes not considered to contain growth		
	N_{2b} Nodes considered to contain growth		
N_3	Fixed nodes		

are brought to the veterinarian for treatment of benign epithelial tumors that have ulcerated as a result of self-trauma or secondary inflammation.

Most benign tumors are well circumscribed, painless, and freely movable and incite a minimal inflammatory response. In contrast, malignant tumors tend to be rapidly growing, fixed to underlying structures, and ulcerated and often have ill-defined margins. Invasion into vessels and regional lymphatics may be observed.

DIAGNOSTIC TECHNIQUES AND WORKUP

Two crucial elements of the diagnosis and management of skin tumors are a thorough history and a detailed physical examination. The history should include questions about the duration of the lesion, rapidity of growth, change in appearance over time, travel history, presence of pruritus, response to previous therapy, and related medical history. Every tumor should be examined for size, location, consistency, presence or absence of fixation to underlying tissue, and whether the overlying skin is ulcerated. Three-dimensional or longest diameter caliper measurements of the tumor, its location, and a digital photograph should become a permanent part of the medical record. A thorough examination of draining lymph nodes also is important.

The physical appearance, location, and growth pattern of a lesion may give the examiner a high degree of suspicion as to the type of tumor involved. However, it is imperative that some type of cytologic or histopathologic diagnosis be obtained so that therapy can be planned properly and the client can be given as accurate a prognosis as possible. The two most common diagnostic procedures for skin tumors are fine-needle aspiration and nonaspiration cytology and tissue biopsy.

Cytology is an important screening tool for differentiating neoplastic from inflammatory lesions (see Chapter 7).[71,72] Virtually all cutaneous tumors should be evaluated by fine-needle cytology to aid the planning of therapy. Romanowsky-type dip stains are quick and inexpensive and should be a part of every practice. Several types of tumors (e.g., well-differentiated mast cell tumors and melanocytic tumors) lend themselves well to cytologic diagnosis. Cytology often allows differentiation of epithelial from connective tissue tumors; however, special training is required for further subclassification of many of these tumors. Cytologic examination of enlarged regional lymph nodes should be performed before definitive therapy. The practitioner must bear in mind that ulcerated or inflamed tumors may cause reactive lymphadenopathy without metastasis.

Histologic examination of a suspected or known tumor is extremely important for planning therapy and devising a prognosis. Histologic examination of an

excised specimen allows the pathologist to determine the degree of malignancy and invasion and whether surgical excision was adequate. The type of biopsy procedure used is dictated by the size and location of the tumor. A small tumor in an easily accessible location that is amenable to adequate surgical margins usually is treated by excisional biopsy. It is important to submit the entire specimen for histologic examination and margin analysis (see Chapter 3). For large tumors or tumors in locations that do not allow easy excision with wide margins (e.g., an extremity), an incisional or punch biopsy should be performed to allow optimal therapeutic planning. Small, flat, or plaquelike skin lesions should not be rigorously prepped or scrubbed before an incisional biopsy to ensure assessment of undisturbed surface pathology.

With some neoplastic processes, particularly cutaneous round cell tumors, the degree of differentiation may not lend itself well to routine light microscopy classification. Immunohistochemical techniques have been explored for their usefulness in differentiating these tumor types.[73-82] Monoclonal antibodies directed at specific tissue components, including intermediate filament proteins (e.g., cytokeratin and vimentin) and surface differentiation antigens, can be useful for classifying poorly differentiated tumors into epithelial, mesenchymal, or lymphoid categories and in some cases specific histologic groupings (examples are discussed under specific tumor types).

Molecular diagnostic techniques (e.g., genomics and proteomics) and advanced assays of molecular events (e.g., apoptosis, proliferation, and kinase receptor expression) are becoming important in many human cancers, both from a diagnostic and a prognostic standpoint (see Chapter 8), and some of these techniques have been applied to cutaneous tumors from veterinary patients (tumor-specific examples are discussed later in the chapter).

Depending on the tumor type in question or the clinician's index of suspicion (or both), an expanded diagnostic workup may be indicated to determine whether systemic spread has occurred and to evaluate the patient's readiness for therapeutic intervention. If the lesion probably is a malignancy, these diagnostics are performed before definitive therapy. Occasionally, with skin tumors amenable to adequate surgical excision in otherwise healthy patients, such diagnostics are performed after excisional biopsy and only if the histologic assessment warrants. The choice of expanded diagnostics often is driven by the known or suspected tumor type. For example, thoracic radiographs and abdominal ultrasound may be warranted for subungual melanoma, whereas bone marrow aspiration is appropriate when cutaneous lymphoma is suspected.

Knowledge of the extent of cutaneous tumor margins before surgery (usually acquired by digital palpation and occasionally local radiographs) may be enhanced by the use of diagnostic ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI) (see Chapter 6). In a study of dogs with cutaneous mast cell tumors or soft tissue sarcomas, the extent of local tumor margins was upgraded in 19% of cases that used US and in 65% of cases that used CT.[83] Such information may allow more appropriate planning of definitive surgery or radiotherapy.

TREATMENT AND PROGNOSIS FOR SPECIFIC TUMOR TYPES

In many cases skin tumors are treated before the specific tumor type is known (e.g., an excisional biopsy often is both diagnostic and curative), therefore the general principles of treatment of skin tumors are discussed collectively. The specific form of therapy is determined by the nature of the primary disease, local and distant metastasis, the anticipated behavior of the tumor, and the patient's overall condition. When only local disease is present, the size and location of the tumor are important in determining the appropriate therapy.

Standard blade excision remains the treatment of choice for most skin tumors. Standard surgical technique is used, with emphasis on adequate surgical margins (see Chapter 10). When the goal is complete excision of a tumor, it is better to leave an open wound, if necessary, than to leave tumor. A major advantage of surgical excision of skin tumors is that the completeness of surgery can be determined histologically. Cytoreductive surgery may be used for very large tumors for palliation or to facilitate other forms of therapy (e.g., radiation, chemotherapy, or immunotherapy). The leading cause of failure of surgical excision is inadequate surgical margins. Amputation should be considered for large malignant tumors on extremities. For mast cell tumors (see Chapter 19) and soft tissue sarcomas (see Chapter 20), cytoreductive surgery followed by radiotherapy offers an effective alternative to amputation, typically leading to long-term local control.

Because most cutaneous tumors are in accessible locations, they lend themselves to a number of treatment modalities, such as cryosurgery, radiation therapy, laser ablation, photodynamic therapy, and hyperthermia.

Cryosurgery may be helpful in the treatment of certain skin tumors (see Chapter 15, section A). The main advantages of this technique are speed, avoidance of general anesthesia in some cases, and low cost (if equipment and cryogen are on site). Cryosurgery is indicated for small or multiple tumors in older animals when anesthesia is a concern. It also is used for small, relatively noninvasive tumors of the nasal planum, pinnae, eyelid, lip, and perianal area. The major disadvantage of cryotherapy is the lack of histologic assessment of "surgical" margins.

Radiation therapy is available on a referral basis in most practice areas of the United States and Canada. It can be used as the primary therapy, as an adjunct therapy for residual tumor control, or sometimes for preoperative cytoreduction. In some cases radiation therapy may produce a better cosmetic result than surgery and may allow retention of anatomic function; however, the cost and repetitive treatment protocols (3 to 5 weeks of treatment) are relative disadvantages.

Other, less established forms of therapy, including hyperthermia (see Chapter 15, section B), lasers, photodynamic therapy (see Chapter 15, section C), intralesional chemotherapeutic implants, immunotherapy (see Chapter 13), and the use of vitamin A–related synthetic retinoids, are showing promise for select groups of tumors (these are discussed with specific tumor types for which efficacy has been investigated).

Systemic and topical chemotherapy also has been used in the treatment of skin tumors. It usually is reserved for cases in which surgical or radiotherapeutic alternatives do not exist or for adjuvant therapy of tumors with a high metastatic or recurrence potential. To date, except for cutaneous lymphoma and mast cell tumors, little is known about the efficacy of this therapy in the treatment of cutaneous tumors.

TUMORLIKE LESIONS

Several types of tumorlike cutaneous and subcutaneous masses are encountered in veterinary practice. These lesions are nonneoplastic but in some instances may mimic neoplastic lesions. The most common nonneoplastic lesions involving the skin of dogs and cats are cutaneous cysts. The most common cysts are epidermoid cysts, dermoid cysts, and follicular cysts.

Epidermoid and follicular cysts often contain gray to white-brown, cheesy material; this material, when expressed through a needle aspiration hole, is the rationale for the nickname "toothpaste tumor." Epidermoid cysts (epidermal inclusion cyst, "sebaceous" cyst) are round to oval, firm to fluctuant, smooth, well-circumscribed lesions common in dogs but rare in cats. They may be solitary or multiple, and although they can be found anywhere in the body, they most often occur on the extremities. These masses may contain gray to white-brown, cheesy material with bits of hair shafts, and they usually are covered by intact epithelium (Figure 18-1). These cysts may become ulcerated or inflamed if cystic contents are extruded into the adjacent tissue. The treatment of choice is surgery or cryosurgery. One dog with multiple epidermoid cysts was treated successfully with oral isotretinoin, one of the vitamin A–related synthetic retinoids.[84]

Dermoid cysts (pilonidal cysts) are similar to epidermoid cysts but have a more complex structure. They appear to be either congenital or hereditary (or both). Breeds that appear to be predisposed to these cysts are the boxer, Kerry blue terrier, and Rhodesian ridgeback.[85,86] Dermoid cysts also have been reported in cats.[87] Dermoid cysts of Rhodesian ridgebacks and their crosses appear to be inherited. They are found on the dorsal
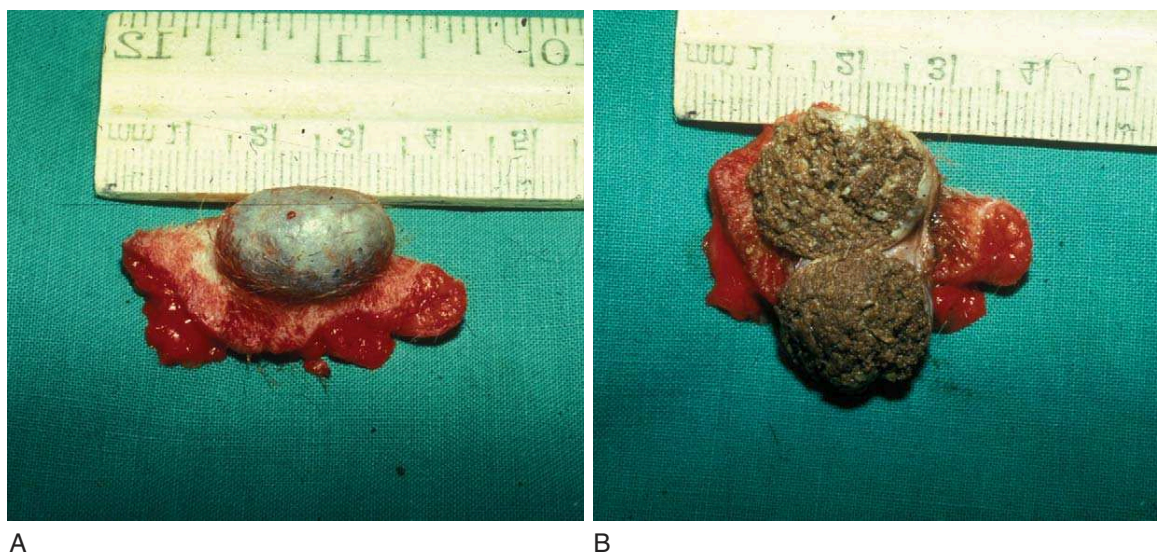
Figure 18-1

Excised epidermoid cyst. **A,** Note the hairless, circumscribed, dome-shaped appearance. **B,** Typical appearance of brown, greasy, granular material inside the cyst.

midline, neck, and scrotum. One case involving the tongue of a dog has been reported.[88] In some cases the lesion may extend deep into the dog's back, to the level of the meninges; therefore these cysts should be evaluated radiographically (e.g., fistulogram and/or myelogram, MRI[89]) to determine whether they communicate with the subarachnoid space. The treatment of choice is surgical excision, and the prognosis generally is excellent.

Follicular cysts are keratinous cysts derived from the epithelium of the outer hair root sheath. They develop as a result of retention of follicular or glandular products caused by obliteration of follicular orifices. They may be congenital or acquired. Follicular cysts, too, may contain gray to white-brown, cheesy material. Therapy is surgical excision, with an excellent prognosis.

Other lesions that may be confused with cutaneous tumors include various forms of keratosis, keratitis (e.g., fungal or foreign body type), xanthomas, calcinosis cutis/circumscripta, and cutaneous mucinosis. Actinic keratosis (solar keratosis) secondary to exposure to sunlight may be a precancerous lesion.[16-18] All these lesions are readily distinguished by histopathologic examination.

Nodular Dermatofibrosis (Collagenous Nevi)

Nodular dermatofibrosis (collagenous nevi) is an unusual, tumorlike syndrome confined to middle-aged German shepherd dogs (mean, 6 years).[41-48] Affected dogs typically have numerous cutaneous nodules that increase in number and location (limbs, head, and trunk) (Figure 18-2). The nodules may become ulcerated, causing lameness. Histologically, these lesions represent hyperplastic dermal collagen (collagenous nevi).

Strong evidence indicates inheritance in an autosomal dominant fashion. Interestingly, in almost all cases the benign skin lesions are associated with multiple bilateral renal cysts, which progress to become cystadenocarcinoma with metastatic potential. Intact bitches with the condition usually develop multiple uterine leiomyomas. Often a long history (months to years) is associated with this condition, and dogs eventually succumb either to renal failure or to widespread metastasis from renal carcinoma. In one large compilation of cases, the mean age at diagnosis of skin lesions preceded the mean age at death from disease by 3 years, attesting to the protracted clinical course.[46] No effective treatment exists, and skin masses are removed only for cosmesis or if they interfere with function.

EPITHELIAL TUMORS

Papillomas

Papillomas (cutaneous papillomatosis, warts, verrucae, squamous cell papilloma) are common skin tumors in the dog but relatively rare in the cat.[5,28-39] They appear as cauliflower-like growths with a finely fissured surface. They may be sessile or pedunculated and when traumatized often bleed. Several types of papillomas are recognized in the dog. In the young dog, papillomas often are multiple in nature and occur most often on the head, eyelids, feet, and mouth (Figure 18-3). These viral papillomatoses are associated with deoxyribonucleic acid (DNA) viruses, which have been reported in the bovine, equine, feline, canine, and human species.[16,25,26,28-39]

Figure 18-2

Nodular dermatofibrosis in a middle-aged German shepherd dog. Note the multiple nodular growths, some ulcerated, on the limbs.

Figure 18-3

Multiple papillomas on the lip and gum of a young dog. These papillomas spontaneously regressed within 3 weeks.

The disease is contagious from dog to dog and has an incubation period of 30 days. Usually no treatment is necessary, because the lesions normally resolve within 3 months. If the lesions affect mastication, surgery or cryotherapy may become necessary. Puppies treated with cyclosporin develop a generalized, nonregressing form of the disease, and one report of the development of cutaneous papillomatosis in a dog undergoing immunosuppressive chemotherapy suggests the importance of an intact immune system in the regression of this disease.[35,90] Biologic response modification with levamisole, thiabendazole, and wart vaccines is for the most part ineffective. Autogenous vaccines have been implicated in the development of injection-site squamous cell carcinoma.[22]

Another type of papilloma often is seen in older dogs. These tumors usually are solitary but may be multiple, and they most often are located on the head, feet, eyelids, and genitalia. Papillomas of the older dog generally are not thought to be associated with a viral etiology. However, newer molecular techniques (e.g., polymerase chain reaction [PCR] and fluorescent in situ hybridization) have thrown this assumption into question, and several reports of dogs with virus-associated multiple cutaneous inverted papillomas have been described.[13-15,25,26,29,36] Some evidence suggests that the canine "oral" papillomavirus may induce nonoral papillomas and ultimately may be responsible for progression to squamous cell carcinoma. Inverted papillomas are the endophytic variant of the viral papillomas that occur in younger dogs; they manifest as cup-shaped lesions with a central core of keratin that leads to an umbilicated surface similar in structure to an intracutaneous cornifying epithelioma.[13,15,91] They normally occur in older dogs, and a papillomavirus distinct from the one that infects young dogs is thought to be involved. A syndrome of multiple papillomas of the footpad of dogs also has been reported but has not yet been associated with a viral etiology. Papillomavirus infection has been associated with multiple pigmented plaques (pigmented cutaneous papillomatosis, pigmented epidermal nevi) of schnauzers and pugs.[30,31,34] In one report, progression or malignant transformation to squamous cell carcinoma was observed.[34] The treatment for solitary papillomas is surgery or cryosurgery, and the prognosis generally is excellent.

Squamous Cell Carcinoma (Epidermoid Carcinomas)

Squamous cell carcinoma (SCC) is a common tumor involving the skin. It accounts for approximately 15% of cutaneous tumors in the cat[1,3,5,6] and 5% of those in the dog.[1-4] Squamous cell carcinomas usually are found in unpigmented or lightly pigmented skin. Many cases involve a recognized relationship to solar exposure, and these tumors often are referred to as *actinic SCC*.[1,16-19]

Figure 18-4

Typical appearance of a red, raised, ulcerated squamous cell carcinoma on the prepuce of a 5-year-old dalmatian. The suture denotes the area of incisional biopsy. Surgical removal resulted in control for over 2 years, at which time a second lesion developed on the opposite flank.

The possibility of an association with papillomavirus and abnormalities in molecular and genetic events has already been discussed.

The most common cutaneous locations for SCC in the dog are the nail bed (see the subungual tumor section later in the chapter), scrotum, nasal planum, legs, and anus. Tumors also have been reported to affect unpigmented or lightly pigmented skin on the flank and abdomen in dalmatians, beagles, whippets, and white English bull terriers (Figure 18-4).[24,92] A histologic variant of SCC, so-called signet-ring SCC, also has been reported in the dog.[93] The most common cutaneous locations for SCC in the cat are the sparsely haired areas of the nasal planum, eyelids, and pinnae (Figure 18-5). Multiple facial lesions are present in nearly 30% of affected cats. SCC affects older animals (mean age of 12 years in the cat, 8 years in the dog). Siamese cats are underrepresented, as would be expected because of their protective pigmented skin color.

SCC may manifest as either a proliferative or an erosive lesion. Proliferative lesions may vary from a red, firm plaque to a cauliflower-like lesion that often ulcerates. The erosive lesion, which is most common in the cat, initially starts as a shallow, crusting lesion that may develop into a deep ulcer. Histologically, the initial crusting lesions often represent carcinoma *in situ* or preinvasive carcinoma (i.e., T_{is} clinical stage; see Table 18-4).

Generally, squamous cell carcinomas involving the facial skin of cats are locally invasive but late to metastasize. The degree of local invasion can be quite severe, and
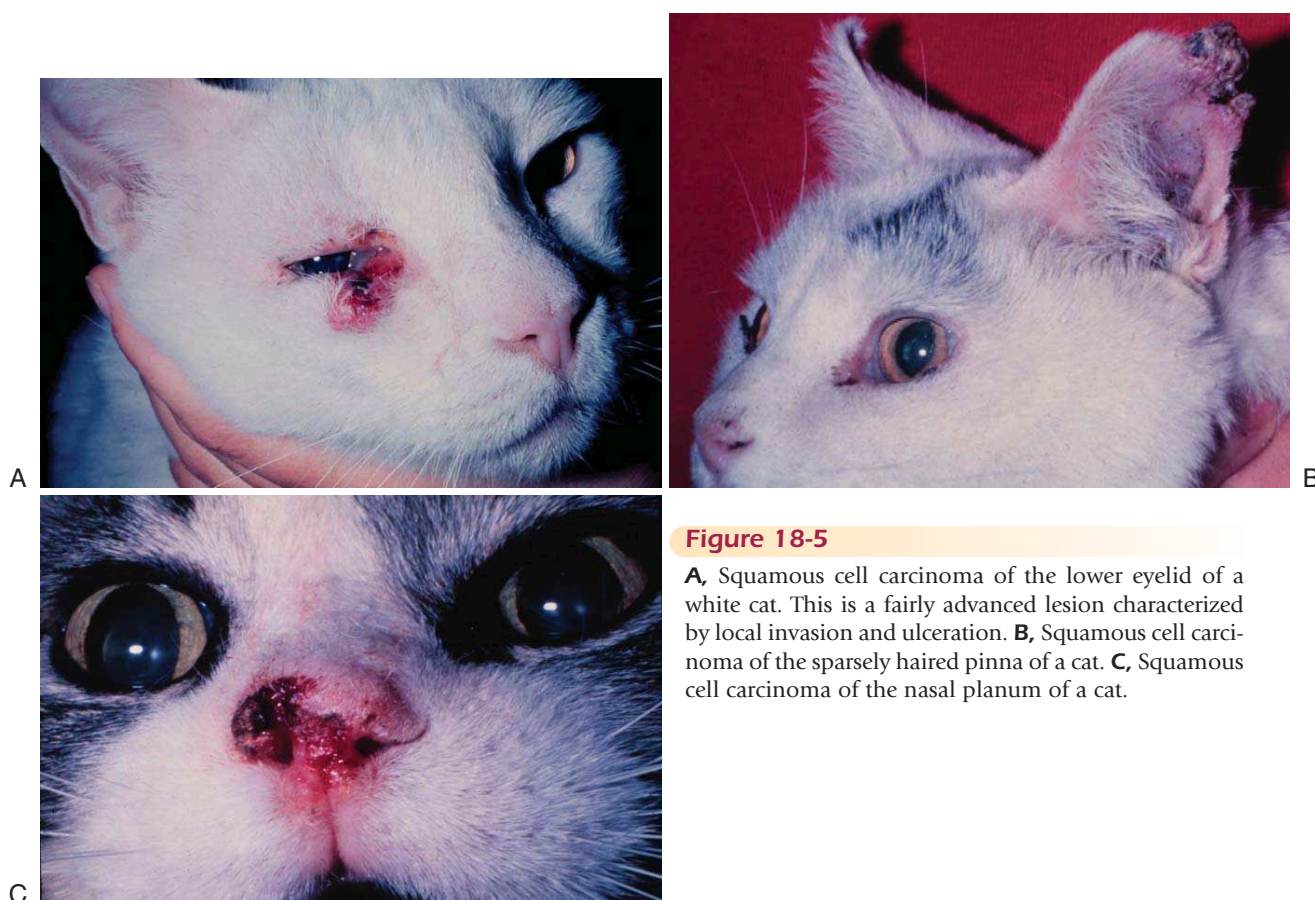
Figure 18-5

A, Squamous cell carcinoma of the lower eyelid of a white cat. This is a fairly advanced lesion characterized by local invasion and ulceration. **B,** Squamous cell carcinoma of the sparsely haired pinna of a cat. **C,** Squamous cell carcinoma of the nasal planum of a cat.

response to therapy is much more successful for T_{is} and T_1 lesions than for those with significant invasion. (The behavior of subungual [nail bed] SCC is discussed at length later in the chapter.) Tumors involving the skin of the flank and ventral abdomen in the dog are locally invasive and have a low metastatic potential; however, multiple lesions often are present throughout the skin of the ventral abdomen, ranging from carcinoma *in situ* to more infiltrative and nodular SCC.

Many therapeutic modalities have been applied to SCC of the facial skin in cats. Most small, early stage lesions seem to respond to most treatment modalities. Surgery and cryosurgery are the mainstays of treatment for these lesions, although numerous reports now detail the use of radiotherapy and photodynamic therapy. The outcome generally is good for most modalities if the tumor is treated early (i.e., T_{is} or T_1). In general, lesions of the pinnae are more manageable than those of the nasal planum because of their location, which allows more aggressive surgical or cryosurgical therapy. Surgical excision of lesions of the pinnae results in long-term control (longer than 1.5 years) in most cases.[94,95] In a report of 102 cats with 163 lesions, aggressive

cryotherapy was nearly 100% effective for managing pinnae and eyelid tumors; however, only 70% of nasal planum tumors responded.[96] (The surgical procedure for infiltrative nasal planum SCC is discussed in Chapter 22, section A). A technique for *en bloc* resection of extensive lower eyelid SCC has been described, involving reconstruction by third eyelid lateral advancement and local transposition of a cutaneous flap.[97]

Several protocols exist for radiation therapy of SCC. Orthovoltage radiotherapy using a total dose of 40 Gray administered in 10 fractions was applied in 90 cats with nasal planum SCC.[98] Overall the 1- and 5-year progression-free survival (PFS) rates were 60% and 10%, respectively. The tumor stage was found to be highly prognostic. The 5-year PFS for T_{is} and T_1 lesions was 56%; higher stage tumors responded poorly. In this report, survival also could be predicted by determining the proliferation fraction of the tumor using a histochemical stain for proliferating cell nuclear antigen (PCNA). Similarly, [90]strontium plesiotherapy, a form of superficial radiotherapy, provided long-term control in 25 cats with early superficial lesions; the 1- and 3-year control rates were 89% and 82%, respectively.[99]

Plesiotherapy is limited to very early, shallow lesions, because the radiation dose drops off significantly below depths of 2 mm. Proton beam radiation therapy, currently a very limited modality, was applied in 15 cats with nasal planum SCC.[100] This treatment resulted in a 93% response rate (60% complete response), with a 64% 1-year control and a median survival of 946 days. As with other tumors, early stage disease was more controllable than late stage disease. In one case, advanced eyelid SCC in a cat was treated with brachytherapy (see Chapter 12) using implanted radioactive [198]gold seeds; a complete response resulted, without recurrence after 10 months of observation.[101] Currently, combinations of surgery and radiation therapy for infiltrative nasal planum SCC are being evaluated and show early promise.

Photodynamic therapy (see Chapter 15, section C) also has been studied extensively for the treatment of superficial SCC in both the dog and the cat.[102-111] If applied to early lesions, the results generally are positive, and long-lived responses are the norm. Complete response rates of approximately 75% to 100% have been reported for T_{is} or T_1 tumors; the rate drops off quickly to below 30% for higher stage tumors.

The use of chemotherapy as a single agent treatment for cutaneous SCC has shown little consistent efficacy in the veterinary literature. Agents that have been investigated on a limited basis for SCC in the dog and cat include mitoxantrone,[112,113] actinomycin D,[114] doxorubicin/cyclophosphamide combinations,[115] bleomycin,[116] and cisplatin (which should not be used in cats).[116–119] Response rates generally are low and short-lived. Recently, a protocol involving the use of intralesional carboplatin in conjunction with external beam radiotherapy was reported in six cats with advanced stage nasal planum SCC (stages II, III, and IV).[120] This protocol used 12 fractions of 4 Gy each for 4 weeks (3 fractions per week) in combination with once weekly intralesional administration of carboplatin (1.5 mg/cm^3 tumor volume) delivered 30 minutes before a radiation fraction. With a median follow-up of 268 days, all cats had a complete response, and four had a durable response (i.e., at least 8 months). This is the only existing report of durable responses for advanced stage disease in cats with nasal planum SCC involving chemotherapy and radiation therapy, although only a small number of cats were involved, and the treatment cohorts need to be expanded.

The use of chemotherapy after surgery as an adjuvant treatment for microscopic disease should also be investigated for high-grade lesions. The use of intralesional sustained-release cisplatin and 5-fluorouracil (5-FU), as well as intralesional carboplatin (alone or with hyperthermia), has been investigated in dogs with superficial SCC.[121-124] Approximately 50% of cats and dogs with actinic-related SCC achieved a complete response with intralesional therapy, many with durable responses.

The vitamin A–related synthetic retinoids also have been evaluated in dogs and cats with solar-induced cutaneous SCC. Only preneoplastic lesions responded to etretinate, and early superficial lesions responded to a combination of isotretinoin and local hyperthermia in the dog.[125,126] No significant response was noted in 10 cats treated with isotretinoin.[127]

The nonsteroidal antiinflammatory cyclooxygenase (COX) inhibitor piroxicam, also known for its immunomodulating effects, has been evaluated for efficacy in dogs with nonresectable SCC.[128] Partial responses were noted in five of 10 patients treated, with a resulting median survival of 150 days. Although no reports of therapy with piroxicam or other COX-2 inhibitors exist for cats with SCC, very low levels of COX-2 immunoreactivity have been reported in cutaneous SCC in cats, which implies that responses would be limited.[129]

Another variation of SCC reported in cats is best referred to as *multicentric SCC in situ* (MSCCIS, Bowen's disease, or bowenoid carcinoma *in situ*).[130-133] Unlike actinic or solar-induced SCC *in situ*, MSCCIS is found in haired, pigmented areas of the skin and is unrelated to sunlight exposure. It has not been associated with either FeLV or feline immunodeficiency virus (FIV) infections. Multiple lesions usually are present in older cats, and lesions are confined to the epithelium with no breach of the basement membrane. Lesions generally are crusty, easily epilated, painful, and hemorrhagic when manipulated. They are thought to be preneoplastic, because three cats had true SCC adjacent to sites of MSCCIS. When excision is possible, recurrence has not been reported; however, similar lesions often develop at other sites. The lesions are not responsive to antibiotics or corticosteroids and are variably responsive to [90]strontium plesiotherapy.

In dogs, infiltrative SCC of the nasal planum (Figure 18-6) is more difficult to manage than in cats.[134-137] Recurrence after surgery and radiotherapy is likely; however, long-term survival has been achieved in a small number of cases after combined resection of the nasal planum and premaxilla, because this allows more aggressive resection than nasal planum resection alone (see Chapter 22, section A, for a description of the surgery).[135,137]

The prognosis for cutaneous SCC in cats and dogs is most strongly predicted by the tumor stage. Other factors that may be predictive are the histologic grade, proliferation rate (e.g., PCNA, Ki-67, silver staining of nucleolar organizer region [AgNOR]), vascular endothelial growth factor (VEGF) expression, apoptotic rate, and nuclear morphometry; however, further investigation is required to establish the predictive power of these factors.[59,60,98,138-140]

Basal Cell Tumors

Basal cell tumors (BCTs) include basal cell epitheliomas, basal cell carcinomas, and basiloid tumors. Because the tumor in domestic animals is almost always benign, the preferred nomenclature is *basal cell tumor*. BCTs are the most common type of skin tumor in the

cat, accounting for 15% to 26% of all feline skin tumors.[1,5,6] These tumors are less common in the dog, representing 4% to 12% of canine skin tumors.[1,2,4] BCTs generally are found in middle-aged dogs (6 to 9 years) and in slightly older cats (mean age, 10 to 11 years).[5,6] Among dogs, cocker spaniels and poodles have a higher incidence; with regard to cats, one large study reported

an overrepresentation of Siamese cats, but other studies have not documented a breed predilection.[5,6]

Basal cell tumors usually are solitary, well-circumscribed, firm, hairless, dome-shaped, elevated masses measuring 0.5 to 10 cm in diameter (Figure 18-7). Most BCTs are freely movable and firmly fixed to the overlying skin, but they rarely invade the underlying fascia. These tumors are most commonly found on the head, neck, and shoulders in both the dog and the cat (Figure 18-8). Feline BCTs may be pigmented and cystic or solid; they occasionally are ulcerated, and they have a surprisingly high mitotic rate for a benign tumor.[6] Most BCTs are benign, grow slowly, and may be present for months before they are diagnosed. Although needle aspiration cytology has been described for BCTs in the dog and cat, the cytologic features were nonspecific and probably would result in underdiagnosis of the tumor.[141] Advanced diagnostic and investigational techniques have been applied to BCTs in dogs and cats, including evaluation of mitotic activity, proliferation rate, and apoptotic rate/control; although differences between BCTs and SCCs have been reported for these indices, none is consistently prognostic.[53,63,140]

The treatment of choice for BCTs is surgical excision, which carries a good prognosis. In 124 cases of BCTs in cats treated by surgical excision, none of the tumors recurred or metastasized.[142] In another report of 97 BCTs in cats, approximately 10% were classified as histologically

Figure 18-6

Infiltrative squamous cell carcinoma of the nasal planum in a dog.

A   B

Figure 18-7

A, Firm, circumscribed, pigmented basal cell tumor on the face of a cat. **B,** Cross section of a benign basal cell tumor from a cat. Note the cystic center and well-defined margins.

Figure 18-8

Large basal cell tumor that had been slowly growing on a dog.

Figure 18-9

Close-up of a sebaceous adenoma before cryosurgery. Note the roughened surface, which often is greasy to the touch.

malignant; however, only one developed metastasis to the regional lymph nodes.[6] Rare recurrences and no metastasis have been reported in the dog. Cryosurgery and photodynamic therapy are alternatives to surgery for smaller lesions (less than 1 cm in diameter).[143]

Sebaceous Gland Tumors

Sebaceous gland tumors represent a complex array of growths, which can be divided into four groups based on histologic appearance. In decreasing frequency, these are sebaceous hyperplasia, sebaceous epitheliomas, sebaceous adenomas, and sebaceous adenocarcinomas. Sebaceous gland tumors are among the most common skin tumors in the dog, accounting for 6.8% to 7.9% of all skin tumors.[1,2,4,144] These tumors are less common in the cat, accounting for 2.3% to 4.4% of all skin tumors.[1,5,6,145] Modified sebaceous glands are found in a variety of locations and may give rise to neoplastic growths, including eyelid meibomian gland tumors (see Chapter 30) and perianal gland tumors (see Chapter 21, section H).

Sebaceous hyperplasia accounts for most sebaceous gland tumors in the dog. These lesions are characterized histologically by an accumulation of nearly mature sebaceous glands. Most are solitary, although multiple lesions can occur. The lesions are found on older animals (mean, 9.1 years), and miniature schnauzers, beagles, poodles, and cocker spaniels appear to be overrepresented.[144] Sebaceous hyperplasia can occur anywhere on the body, but most lesions are found on the limbs, trunk, and eyelids. Most are less than 1 cm in diameter, are wartlike or cauliflower-like, and can become ulcerated as a result of trauma (Figure 18-9). In a compilation of 92 cases in the dog, only one lesion recurred after excision; however, nearly 10% of the dogs developed new lesions at other body sites.[144] Sebaceous hyperplasia often is found peripheral to and phasing into sebaceous adenomas or adenocarcinomas and is likely a precursor to their development.

In the cat, sebaceous hyperplasia typically manifests as a solitary lesion, often on the head, with a male predisposition.[5,87,145] Lesions may be present for many years. Recurrence has not been reported after excision.

Sebaceous epithelioma occurs primarily on the head (especially the eyelid) as solitary lesions, although generalized cases have been reported. Shih Tzus, Lhasa apsos, malamutes, Siberian huskies, and Irish setters appear to be overrepresented. The lesions are nearly identical in appearance to those of sebaceous hyperplasia, and the treatment of choice is excision. A sebaceous epithelioma is more likely to recur than is sebaceous hyperplasia or a sebaceous adenoma, but the recurrence rate is still low, approximately 6%.[144]

Sebaceous adenomas are relatively uncommon sebaceous gland tumors that are similar in appearance and
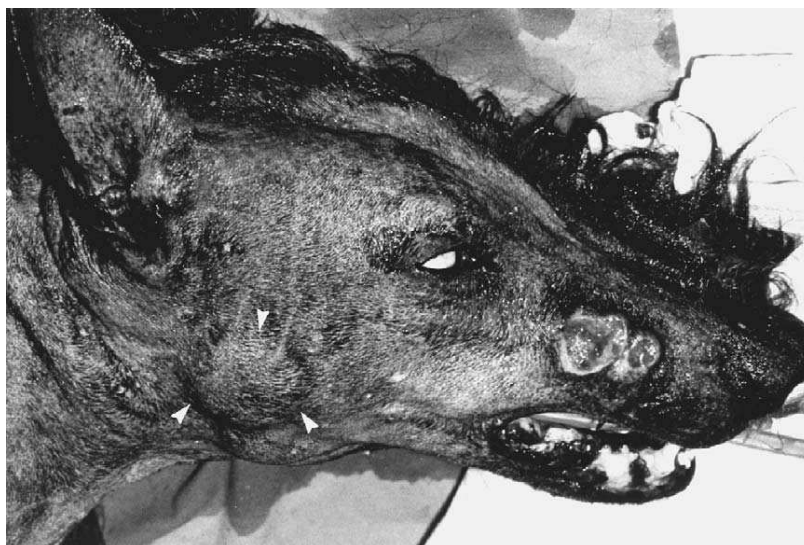
Figure 18-10

Scottish terrier with a sebaceous gland adenocarcinoma of the upper lip with metastasis to the regional lymph nodes *(arrows)*.

behavior to hyperplastic lesions. Sebaceous adenocarcinomas are rare in the cat and dog and appear to have a low potential for metastasis and recurrence.[5,144-146] They are characterized by ulceration and inflammation of surrounding tissues (Figure 18-10).[147] More aggressive surgical excision is indicated for these tumors.

Sweat Gland Tumors

Sweat gland tumors can derive from either apocrine glands or eccrine glands. Eccrine-derived tumors are rare in the dog and cat, and occur only on the footpad. The one report in cats describes lameness and multiple digit involvement, and one of two cats had pulmonary metastasis at the time of diagnosis.[148]

Apocrine sweat gland cysts, which are benign, tumorlike lesions, are more common in the dog. They appear as elevated, round, fluctuant, intradermal nodules that contain a clear, watery liquid (Figure 18-11). They usually are found on the head, and they are easily managed by surgical excision. They have been reported to occur as multiple cysts on the eyelids of oriental breed cats.[149,150]

Tumors of the apocrine sweat glands (adenomas and adenocarcinomas) are rare in the dog, accounting for approximately 2% of canine skin tumors.[151] They are more common than sebaceous gland tumors in the cat, accounting for 3.6% to 9% of skin tumors in this species.[5,6,151] Adenocarcinomas appear to predominate,

Figure 18-11

Apocrine sweat gland cyst on a dog.

representing 50% to 90% of cases in both the dog and the cat. Although most tumors can be differentiated by routine light microscopy, immunohistochemistry has been used to distinguish between the adenoma and adenocarcinoma.[152] One clear cell histologic variant has been reported in the dog.[153]

Sweat gland tumors usually occur in older dogs (median, 9 years).[154] In the dog, these tumors can occur anywhere on the body, although most develop on the limbs. They usually are solitary, raised, well circumscribed, and solid. Nearly half are ulcerated, and the average size is 2 cm in diameter, according to the

largest report.[154] Histopathologic evidence of lymphatic infiltration is seen in 25% of cases, but distant metastasis is uncommon. In one report, only one in 25 dogs had distant metastasis, and this animal was the only one with histologic evidence of vascular invasion.[154] Combining two series, a median postexcision survival of 30 months was reported.[151,154] The efficacy of radiotherapy for unresectable tumors and of chemotherapy for metastatic lesions has not been established.

In the cat, benign apocrine sweat gland adenomas rarely ulcerate. They are associated with little local inflammation and have a cystic feel on palpation. Adenocarcinomas are more likely to be ulcerative, firm, and locally inflamed.[151] Adenomas occur more often on the head, whereas adenocarcinomas can occur anywhere on the body. They occur primarily in older cats (average age, 12 years). Wide surgical excision is the therapy of choice; it carries a good prognosis for adenomas and a guarded prognosis for adenocarcinomas. Local recurrence has been reported. Widespread metastasis, although uncommon, has been reported to local lymph nodes, the digits of the feet, the liver, and the lungs. A single report describes multiple primary digital apocrine sweat gland carcinosarcoma in the cat.[155]

Two modified apocrine gland tumors are discussed at length elsewhere; these are tumors of the ceruminous glands of the ears (see ear canal tumors later in this chapter) and tumors of the anal sac (see Chapter 21, section H).

Intracutaneous Cornifying Epithelioma (Keratoacanthoma)

Intracutaneous cornifying epithelioma (ICE) is a benign epithelial proliferation that arises from the superficial epithelium between hair follicles, although it may appear to originate from the adnexa. These lesions sometimes are included in discussions of hair follicle tumors.[84,156] Only one case in the cat has been reported in the veterinary literature. Canine ICE can manifest in two distinct forms: (1) as a solitary lesion, which may occur in any breed, and (2) as a multicentric form, which usually occurs in Arctic Circle breeds (e.g., Norwegian elkhound and keeshond) (Figure 18-12). Young male dogs appear to be at increased risk. The cause of ICE is unknown, although some evidence suggests the influence of genetic factors in the multicentric form.

Most tumors are 0.5 to 4 cm in diameter and have a pore opening to the surface. The pore usually contains a mass of keratin, which sometimes contains hair shafts. Like epidermoid cysts, these tumors often have a cheesy material that can be expressed manually (i.e., toothpaste tumor). Rupture of the cyst and extrusion of the contents into adjacent tissue may lead to a sometimes marked secondary inflammatory response. In some cases ICEs may be located in the dermis and

Figure 18-12

Multiple intracutaneous cornifying epitheliomas (keratoacanthomas) in a Kerry blue terrier.

subcutaneous tissue without communication to the surface. Cytologically, these tumors are characterized by keratinous debris, clusters of squamous cells, and occasionally cholesterol crystals.

The treatment for solitary tumors is surgical excision, and the prognosis generally is excellent. Multiple small tumors may be treated with cryosurgery. Modest success has been reported in treating the multicentric form of the disease with vitamin A–related synthetic retinoids.[84,156] Both isotretinoin and etretinate have been used successfully, although the latter appears to be more effective. Long-term retinoid therapy is required, and lesions may recur upon discontinuation of treatment. In seven cases of multiple, generalized ICEs, five complete responses and two partial responses occurred.

Tumors of the Hair Follicles (Hair Matrix Tumor)

Tumors of the hair follicles account for approximately 5% of all skin tumors in the dog.[157,158] They are extremely rare in the cat, accounting for fewer than 1% of all skin tumors.[5,6,159] Histologically distinct types, in decreasing order of frequency, include trichoblastomas, trichoepitheliomas, and pilomatrixomas. These make up the majority of cases, although two rare forms, tricholemmomas and trichofolliculomas, also exist.

Trichoblastomas arise from the primitive hair germ epithelium and previously may have been classified as basal cell tumors.[158] Trichoepitheliomas arise from the follicular sheath; these intradermal masses are almost always solitary, round to oval, well-circumscribed, and 1 to 20 cm in diameter. The overlying skin may be atrophic and hairless and often is ulcerated from trauma.

Cut section reveals small, gray-white, multiple foci separated by a thin connective tissue stroma. Trichoepitheliomas occur in older animals, and poodles, golden retrievers, basset hounds, and German shepherd dogs may have an increased relative risk. A histologically unique form with mucinous degeneration is reported to occur primarily in the golden retriever.[157] Most trichoepitheliomas occur over the dorsal lumbar and lateral thoracic region.

Pilomatrixomas derive from hair matrix and account for approximately 20% of hair follicle tumors in the dog. Miniature poodles and Kerry blue terriers are at increased relative risk. These lesions are solitary, solid tumors that range from 1 to 10 cm in diameter. The skin overlying them is hairless and often ulcerated and on cut surface examination may be gritty as a result of mineral deposition. Approximately 25% of tumors may be cystic and 33% hyperpigmented. Needle aspiration cytologic samples have been described as consisting primarily of basaloid cells in association with "ghost cells."[160]

In general, hair follicle tumors are benign and have an excellent prognosis after surgical excision. In a compilation of 80 cases, no local recurrences or metastasis was recorded, even though many of the lesions had histologic evidence of malignancy.[157] A single case report described a dog with multiple benign pilomatrixomas that responded to treatment with isotretinoin.[161] Two reports of metastatic pilomatrixoma exist in the dog, both showing neurologic involvement.[162,163] The author has seen three cases of pulmonary metastasis from malignant pilomatrixomas. Recently, a series of 26 cases of a clear cell adnexal carcinoma in dogs was reported.[164] (Lesions of this type previously were called *clear cell hidradenocarcinoma* and *follicular stem cell carcinoma*.) In this series, recurrence occurred in one case, metastasis to regional lymph nodes occurred in two cases, and pulmonary metastasis may have developed in one case.

MESENCHYMAL TUMORS

Mesenchymal tumors arise from connective tissues. The most common mesenchymal tumors of the skin and subcutis in the dog are mast cell tumors (see Chapter 19), lipomas, soft tissue sarcomas (see Chapter 20), melanomas, and histiocytomas (see Chapter 32, section F).[1-4] The most common mesenchymal tumors in the cat are mast cell tumors (see Chapter 19) and fibrosarcomas (see Chapter 20).[1,3,5,6]

Dermal Fibropapillomas in Cats (Feline Sarcoid)

Several reports of cutaneous fibropapillomas (sarcoid) in cats exist.[37,38,165,166] Clinically, these tumors usually are solitary (rarely multiple), firm, alopecic, and often focally ulcerated masses that occur most often on the head (nose, lip, and pinna), neck, or digits. They are most common in young cats (median, 4 years; range, 3 months to 15 years). Histologically, cutaneous fibropapillomas are composed of a proliferation of dermal fibroblasts covered by a hyperplastic epidermis with long rete pegs. As with equine sarcoids, an association with papillomaviruses has been documented with this disease in cats. Local recurrence occurs after surgical excision in approximately 30% to 50% of reported cases, but metastasis has not been documented.

Cutaneous Smooth Muscle Tumors

Cutaneous smooth muscle tumors are rare tumors that occur in both dogs and cats. They arise from the arrector pili muscles or smooth muscles of the dermal vasculature.[167] In decreasing order of frequency, they include piloleiomyomas, piloleiomyosarcomas, angioleiomyosarcomas, arrector pili hamartomas, and angioleiomyomas. They can recur after incomplete excision, but metastasis has not been reported.

ROUND CELL TUMORS

All round cell tumors can involve the skin and subcutis. Cytologically, these tumors are characterized by discrete round cells. The six types of round cell tumors primarily encountered in the skin and subcutis are histiocytomas, mast cell tumors, plasma cell tumors, lymphoma, transmissible venereal tumors (TVTs), and neuroendocrine (Merkel cell) tumors. In their poorly differentiated form, many of these tumors (especially mast cell tumors, lymphoma, plasma cell tumors, and histiocytomas) can be difficult to characterize by routine light microscopy, therefore specific histochemical and immunohistochemical stains sometimes are used.[74-81,168] An algorithm for the differential diagnosis of these four tumor types has been suggested (Figure 18-13).[81] Detailed discussions are presented elsewhere for mast cell tumors (see Chapter 19), cutaneous lymphoma (see Chapter 31, sections A and B), cutaneous plasma cell tumors (see Chapter 31, section D), TVTs (see Chapter 32, section C), and histiocytomas (see Chapter 32, section F).

Melanocytic Tumors (Melanocytic Nevus, Melanocytoma, Melanosarcoma, Malignant Melanoma)

Tumors of melanocytes and melanoblasts are relatively common skin tumors in the dog, accounting for 5% to 7% of canine skin tumors.[1-4] They are rare in the cat (0.8% to 2.7% of feline skin tumors).[1,5,6] Melanocytic tumors are most common in older dogs (average age, 9 years) that

Figure 18-13

Recommended panel of adjunct stains for the diagnosis of some canine cutaneous round cell tumors. *(From Fernancez NJ, West KH, Jackson ML et al: Vet Pathol 42:437-445, 2005.)*

have darkly pigmented skin, although the literature varies on which breeds are at risk. Early reports mentioned a male preponderance for tumor development, but more recent reports do not support that finding. Melanocytic tumors are also more common in older cats (average age, 10 to 12 years), and no gender or breed predilection is known. The etiology of melanocytic tumors in dogs and cats is largely unknown; however, several investigations into the molecular and genetic basis of melanoma were discussed previously in this chapter and are reviewed elsewhere (see Table 18-3).[55-58,61,66,69,169]

Because these tumors arise primarily in areas of haired skin or in the oral cavity, the causative association with ionizing sunlight is not a factor in these species. Cutaneous melanomas in the dog can be behaviorally benign or malignant and can occur anywhere on the body. These tumors usually can be diagnosed by simple fine-needle aspiration cytology; however, histologic examination is important to determine the potential for malignancy.

Benign forms are often referred to as *melanocytic nevus*, a term that in its purest sense implies any congenital,

melanin-pigmented lesion. Benign melanomas typically are well defined, deeply pigmented, less than 2 cm in diameter, dome shaped, firm, and broad based but mobile over underlying tissue (Figure 18-14). Behaviorally malignant melanomas tend to grow rapidly, can be larger than 2 cm in diameter, and often are ulcerated (Figure 18-15). A summary of factors known to be prognostic for cutaneous melanoma in dogs is presented in Table 18-5. In dogs, more than 85% of melanomas that arise from haired skin are associated with benign behavior. Most oral and mucocutaneous junction melanomas (except the eyelid) and approximately 50% of melanomas arising in the nail bed (see subungual tumors later in the chapter) are behaviorally malignant.[170,171]

Most melanomas can be diagnosed cytologically (Figure 18-16); however, tissue biopsy is recommended, because the mitotic rate, a histologic criterion, is highly predictive of the degree of malignancy (approximately 90% accurate). A mitotic rate of less than 3 per 10 high-power fields is strongly associated with benign behavior.[1,170,171] Highly proliferative melanomas, as measured by Ki-67 or PCNA immuno-histochemical analysis, have been associated with a

Figure 18-14
Typical appearance of a raised cutaneous melanoma. In the dog, most melanomas that occur on haired skin are benign.

Figure 18-15
Behaviorally malignant cutaneous melanoma on the side of a Labrador retriever. The malignant variety of cutaneous melanoma typically is larger, poorly circumscribed, ulcerated, and grows rapidly.

TABLE 18-5	Known or Suspected Prognostic Factors for Malignant Melanoma in the Dog	
Factor	**Comments**	**References**
Location	Tumors arising from haired skin generally are benign. Tumors arising from mucocutaneous junctions (except the eyelid) and nail bed, as well as oral lesions, generally are malignant.	170-172
Histology	Histologic criteria of malignant versus benign are very predictive of biologic behavior. Determination is based primarily on the mitotic index; however, lymphatic invasion also is predictive.	1, 170, 171, 173, 226
Breed	Melanomas are more likely to be benign in the Doberman pinscher and miniature schnauzer and more likely to be malignant in the miniature poodle.	170
Tumor cell proliferation rate	Histochemical and immunohistochemical techniques (e.g., MIB-1/Ki-67, PCNA) that measure proliferation have been shown to be prognostic. However, they are only modestly more predictive than simple histology and the mitotic index.	64, 172, 173
DNA ploidy	Prominent G_2/M peaks are predictive for malignant behavior. However, this technique is no more predictive than simple light microscopy, therefore it is not cost-effective.	170
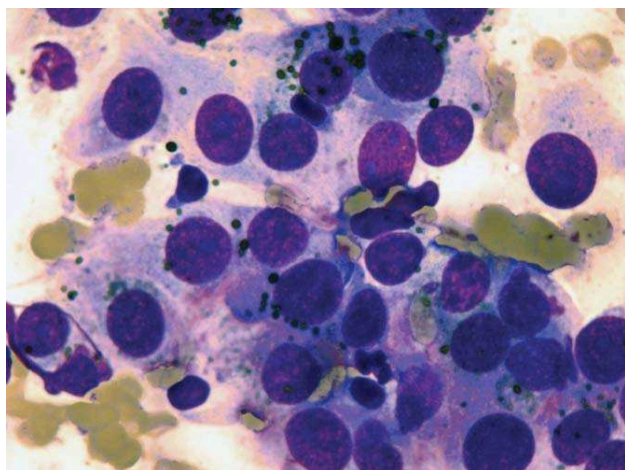
Figure 18-16

Typical cytologic appearance of a melanoma, showing a monotonous, round to spindle cell population with darkly pigmented granules (hematoxylin and eosin (H&E) stain, 100× objective).

more malignant course, but these more advanced procedures offer little more predictive value than the mitotic rate.[64,172,173] Tumors that are grossly infiltrative to underlying structures also represent a more malignant variety.[172]

Breed has been reported to be prognostically significant; more than 75% of melanomas in Doberman pinschers and miniature schnauzers are behaviorally benign, whereas 85% of melanomas in miniature poodles are behaviorally malignant.[170] Analysis of DNA ploidy with flow cytometric analysis strongly correlates with the degree of malignancy for melanoma in the dog,[170] but it is no more predictive than routine light microscopy and therefore is not cost-effective. This relatively time-consuming technique is not clinically useful as a prognostic indicator, but it may be useful for differentiating amelanotic melanomas from other poorly differentiated tumors. Expression of p53 and several other tumor suppressor genes has been evaluated on a limited basis in canine and feline melanomas, but it does not appear to have significant predictive value for outcome.[57,174]

Amelanotic melanomas can occur at cutaneous sites, although they are much more common in the oral cavity of dogs. Special histochemical and immunohistochemical stains may be helpful for diagnosing these agranular variants.[175-177] Immunohistochemical stains also have been used in the differentiation of melanomas from pigmented BCTs in cats.[178] Balloon, signet-ring, clear cell melanocytoma-acanthoma, and pilar neurocristic histologic variants of cutaneous melanoma have been reported.[177,179-181] The prognostic

significance of these rare tumors is largely unknown. In one study, no recurrence or metastasis developed after excision of four clear cell melanomas.[181]

In the cat, melanogenic tumors also may be benign or malignant. Although they can be induced experimentally with the feline sarcoma virus, this etiology is unlikely to be associated with clinically observed cases.[5] Melanogenic tumors most often involve the head (e.g., the nose and pinna) and less commonly the extremities.[5,182,183] Most are ocular or on the eyelid (see Chapter 30). Nonocular melanomas in cats are similar in appearance to those in the dog; however, histologic assessment of malignancy does not appear to predict the clinical outcome, as it does in the dog.[183] In general, ocular melanoma is behaviorally more malignant than oral melanoma in the cat, and dermal melanomas can have a benign or a malignant clinical course.[183-185]

The treatment of choice for local cutaneous melanoma in both the cat and dog is surgical excision. In dogs, tumors with benign prognostic criteria (see Table 18-4) have an excellent prognosis after complete excision. The prognosis for tumors with malignant criteria is guarded; metastatic rates of 30% to 75% have been reported.[170,171] In the cat, the prognosis for nonocular dermal melanomas is fair; recurrence and metastatic rates of 5% to 50% have been reported.[5,182,183] Alternatives to blade excision for local disease include radiotherapy, local hyperthermia and intralesional cisplatin/carboplatin,[122] and photodynamic therapy.[103,104] Coarse fraction radiotherapy has been used successfully for local control of oral melanomas in dogs (see Chapter 21, section A), and it is likely to be beneficial for dermal melanomas when surgery is not an option. However, most dogs with malignant disease succumb to systemic spread. The response to hyperthermia/intralesional cisplatin and photodynamic therapy appears to be short-lived.

Systemic chemotherapy for malignant melanoma in the dog has shown little promise in the veterinary literature. Agents that have been investigated on a limited bases, and primarily for oral melanoma, include mitoxantrone,[112] doxorubicin,[117] dacarbazine,[186] and carboplatin.[187] In general, response rates have been low and the durations of response have been short-lived.

Because of the absence of efficacious chemotherapeutic regimens for metastatic or unresectable melanomas, several novel therapeutic modalities have been investigated. These include methods of enhancing immunosurveillance (e.g., tumor vaccines) and immune-mediated killing of tumor cells, as well as several techniques to target the tumor cells at the molecular level (see Chapter 13).[188-197] Many of these studies were conducted as translational modeling for therapies in both veterinary and physician-based oncology, and although most were for oral malignant melanoma, responders with cutaneous or digital

melanoma have been reported. Immunomodulation therapy currently is an active area of research for malignant melanoma in both physician- and veterinary-based oncology.

Cutaneous Neuroendocrine (Merkel Cell) Tumors

Merkel cell tumors are rare tumors in dogs and cats. They typically are solitary and can occur on the lips, ears, and digits and in the oral cavity. Merkel cells are part of the diffuse neuroendocrine cell system and are believed to function as mechanoreceptors. Histochemical stains and electron microscope morphology are necessary for diagnosis.[168,198-201] In the few reports in the veterinary literature, these tumors appear to be behaviorally benign in the dog and are successfully managed by surgical excision.[3] Only two cases have been reported in the cat.[200,201] In one case, the lesion behaved as a benign tumor after resection; in the other case, multiple pulmonary metastases developed within 2 months of surgical resection.

EAR CANAL TUMORS IN DOGS AND CATS

Tumors of the ear canal are discussed as a group for convenience and clarity. Both benign and malignant neoplasms occur with some frequency in the ear canal of both dogs and cats, and large compilations of cases have been reported.[5,202-204] Although inflammation may occur secondary to tumor development in the area, the presence of longstanding otitis externa is believed to be a factor in tumor development, and observed increased glandular dysplasia suggests that chronic inflammation does indeed play a role. Ear tumors occur in older cats (mean age, 7 years for benign tumors and 11 years for malignant tumors) and dogs (mean age, 9 years for benign tumors and 10 years for malignant tumors). No gender predilection exists in either species. The cocker spaniel appears to be overrepresented for both benign and malignant tumor development, which may be related to this breed's propensity to develop otitis externa.

The most frequently observed clinical presentations include presence of a mass, aural discharge, odor, pruritus, and local pain (Figure 18-17). These signs often are present for months to years before the animal is brought to the veterinarian for treatment. Neurologic signs (e.g., Horner's syndrome, vestibular signs) are observed in approximately 10% of dogs with malignant tumors and 25% of cats with either benign polyps or malignant tumors. Benign tumors are raised, pedunculated, and rarely ulcerated, whereas malignant tumors are more likely to be ulcerated and hemorrhagic and have a broad base of attachment. Approximately 25%

Figure 18-17

Squamous cell carcinoma in the ear canal of a cat, manifesting with discharge, hemorrhage, and odor from the ear canal.

of malignant forms show evidence of bulla involvement, and skull radiographs are recommended as part of the initial diagnostic workup. Three cases of bilateral ear canal tumors have been reported; one ceruminous gland carcinoma and two squamous cell carcinomas.[205]

The most common benign tumors encountered in both species are inflammatory polyps, papillomas, BCTs, and ceruminous gland adenomas. Inflammatory polyps in cats are discussed in detail in Chapter 21, section A. A ceruminous gland cyst is a tumorlike lesion that occurs in cats. These lesions are sessile masses, often multiple, usually purple or black, that contain an oily black fluid (Figure 18-18). They can be mistaken clinically for melanomas or BCTs.

The most common malignant tumor encountered in the dog is ceruminous gland adenocarcinoma (CGA), followed by SCC and carcinoma of unknown origin.[202,203,206] A report of 53 malignant ear canal tumors in cats indicated that CGA and SCC are equally represented as the most common malignancy; however, other reports have indicated that CGA is far more likely to occur than SCC.[5,202,203] Almost every other type of cutaneous tumor has been reported from time to time at a lower incidence in the ear canals of cats and dogs.

Figure 18-18

Multiple benign ceruminous gland cysts in the ear canal of a cat. The cysts had been present and growing slowly for several years.

Malignant ear canal tumors are less aggressive in dogs than they are in cats. Survival data have been generated in dogs and cats with malignant ear canal tumors treated almost exclusively by surgical excision.[202] Most dogs live longer than 2 years after surgery; in cats, the median survival time is 1 year. In general, ear canal tumors are locally invasive and have relatively low metastatic potential. Approximately 10% of dogs and 5% to 15% of cats have evidence of local lymph node or thoracic metastasis at initial diagnosis. Cats with SCC of the ear canal (rather than the pinna) are more likely to have neurologic signs than with other tumor types, which reflects a more invasive behavior. In the dog, bulla involvement and conservative surgery both have been identified as negative prognostic indicators.[202,207] In dogs, aggressive ear canal ablation with lateral bulla osteotomy results in a median survival time of 36 months, compared to 9 months for dogs treated by more conservative lateral ear canal resection. In the cat, the presence of neurologic signs, histopathologic typing of SCC or carcinoma of undetermined origin, histopathologic evidence of lymphatic or vascular invasion, and conservative surgery have been identified as negative prognostic indices. In cats with CGA, a median disease-free interval (DFI) of 42 months, a 25% recurrence rate, and a 75% 1-year survival rate can be expected after aggressive ear ablation and bulla osteotomy. This compares favorably with a 10-month DFI, a 66% recurrence rate, and a 33% 1-year survival after conservative lateral ear resection.[208]

Most benign ear canal tumors in dogs and cats can be readily managed with conservative surgical resection. Evidence indicates that aggressive excision, including ear canal ablation and lateral bulla osteotomy, should be the treatment of choice for malignant ear canal tumors in both species, because tumor invasion into the bullae is a negative prognostic indicator. This type of procedure produces a good prognosis with a high likelihood of long-term survival in the dog. In the cat, a fair prognosis is warranted for CGA and a guarded prognosis for SCC or carcinoma of undetermined origin.[202,208] Radiation therapy is a possible alternative to surgery and can be used as an adjuvant to incomplete resection. In one report, a median progression-free interval of 40 months and a 56% 1-year survival were achieved for five dogs and six cats with CGA that were treated with 48 Gray external beam radiotherapy after incomplete surgical excision.[209] The ear canal also is a location that lends itself to photodynamic therapy (see Chapter 15, section C). Currently, no information exists on the efficacy of chemotherapy for ear canal tumors in the dog and cat.

SUBUNGUAL (NAIL BED) TUMORS

Primary subungual tumors are common in the dog and rare in the cat. A number of large case compilations have been done on tumors in this location.[171,210-216] Approximately one third to one half of subungual tumors in the dog are SCCs, followed in frequency by malignant melanomas, osteosarcomas, various soft tissue sarcomas (fibrosarcomas, neurofibrosarcomas), and mast cell tumors. No breed or gender predilection has been reported. However, in one large compilation, 25% of dogs with malignant nail bed tumors had a black hair coat. In the cat, primary nail bed tumors are rare (these are almost always SCCs), but metastatic nail bed tumors are more common and usually are metastatic lesions of carcinomas in other sites.[155,214,215,217-220] In particular, bronchiolar adenocarcinoma, pulmonary and cutaneous SCC, and apocrine sweat gland carcinosarcoma all have been documented to metastasize to multiple digits in the cat. Bronchogenic adenocarcinoma also can metastasize to other cutaneous sites in the cat.[221,222] Many other types of cutaneous tumors have been reported at this site at a lower incidence.

Tumors comprise approximately 12% of all disorders of the nail and nail bed and should be included in any differential list for disease in this area. Typical presenting signs in dogs and cats with subungual tumors include the presence of a mass, lameness, and ulceration (Figure 18-19, *A*).[216] Respiratory signs are rare in cats with digital metastasis from pulmonary tumors.[214,215] Radiographs of the affected digit should be a routine part of the workup for nail bed disease, because approximately 75% of primary digital tumors result in local bone lysis (Figure 18-19, *B*). If a malignancy is suspected or multiple digits are involved, thoracic radiographs are

Figure 18-19

A, Squamous cell carcinoma of the second digit in a dog. Complete digital amputation was curative.
B, Radiograph of the same dog showing a subungual squamous cell carcinoma of the second digit. Note the almost complete osteolysis of P_3 with periosteal reaction of P_2 and the presence of a soft tissue mass in the surrounding tissues.

also indicated, particularly in the cat. Although benign or infectious processes (e.g., pododermatitis) of the digit can result in local bone lysis, this is much less likely to occur.[212,213] Subungual tumors often are secondarily infected and initially misdiagnosed as chronic paronychia or osteomyelitis. A prolonged history before diagnosis may result.

Subungual SCC in the dog arises from the subungual epithelium and is locally invasive, almost always resulting in bony lysis of the third phalanx. It occurs in older dogs (mean age, 9 years), and no gender predilection is known. Approximately 75% of cases involve large breed dogs, and more than two thirds of these lesions occur in dogs with primarily black hair coats (e.g., black Labrador retriever and standard poodles), as opposed to

the more common development of SCC in lightly pigmented cats and dogs in other sites. The lesions usually are solitary, ulcerative, occasionally hemorrhagic, and expansile (see Figure 18-19). The associated nail may be fractured or absent altogether. Subungual SCC is locally invasive and has a low metastatic potential, contrary to previous reports. In several reports, radiographic evidence of distant metastasis is uncommon (0 to 15%), and regional node or distant metastasis after complete excision (most involving wide digital amputation to the level of P1) develops in approximately 10% to 29% of cases.[210,212,216]

Some evidence suggests that SCC that develops from the subungual epithelium is less malignant than SCC that originates in other parts of the digit. One-year and 2-year

survival rates of 95% and 75%, respectively, were observed in SCC arising from the subungual epithelium, which compared favorably to rates of 60% and 40% for SCC arising from other parts of the digit.[212] The rate of local recurrence rises if local excision is more conservative, therefore treatment for subungual SCC should include a disarticulation amputation at the metacarpophalangeal, metatarsophalangeal, or proximal interphalangeal level. Adjuvant therapy does not appear to be required in most cases. A syndrome of multiple digital SCC in dogs has been reported.[216,223,224] One report involved a standard poodle and a giant schnauzer, another involved three related giant schnauzers that developed SCC in multiple digits over several months to years.

Subungual melanomas are potentially malignant in the dog. Approximately one third to one half of melanomas that originate in the nail bed develop distant metastasis to the lymph nodes, lungs, and other systemic sites.[171,212,216] Digital amputation usually controls local disease (local recurrence rates of 30% can be expected), but approximately half of dogs die as a result of distant metastasis. Effective adjuvant systemic therapy apparently is necessary in most cases; however, as previously discussed, no consistent adjuvants exist for malignant melanoma, and a fair to guarded prognosis is warranted.

Soft tissue sarcomas involving the nail bed appear to behave similarly to their counterparts at other cutaneous sites (see Chapter 20); that is, they are locally aggressive but uncommonly metastatic. Of four digital osteosarcomas, one developed distant metastasis.[216] Four cases of "mesenchymal tumor of undetermined histogenesis" were described in dogs; two of these lesions originated in the nail bed.[225] Long-term survival with local control was achieved after surgical excision. Mast cell tumors (see Chapter 19) of the nail bed (not digital skin) in the dog typically are high-grade, poorly differentiated tumors that have a poor prognosis, similar to mast cell tumors at other mucocutaneous sites.

The prognosis is very poor for cats with metastatic digital tumors, with a median survival time of approximately 1.5 months reported.[214,215] Digital amputation rarely was palliative, because metastasis to other digits occurred quickly. Primary SCC of the digit in cats has a better, albeit guarded, prognosis, because this tumor usually manifests as a solitary mass, and palliation with surgery is more likely. However, in the small number of cases that included follow-up (six), the median survival time still was only 3 months.[214]

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Mast Cell Tumors

Douglas H. Thamm and David M. Vail

The neoplastic proliferation of mast cells, referred to as *mast cell tumor* (mast cell sarcoma), is the most common cutaneous tumor in the dog and the second most common cutaneous tumor in the cat.[1-5] Systemic forms of the disease often are referred to as *mastocytosis*. Canine and feline forms of the disease are considered separately in this chapter, because many differences exist with regard to histologic type, biologic behavior, therapy, and prognosis.

CANINE MAST CELL TUMORS

Incidence and Risk Factors

As mentioned previously, mast cell tumors (MCTs) are the most common cutaneous tumor in the dog, accounting for 16% to 21% of cutaneous tumors.[1,3,5,6] MCTs are primarily a disease of older dogs (mean age, 9 years) but have been reported in dogs ranging from 3 weeks to 19 years of age.[7,8] Most occur in mixed breeds, although boxers, Boston terriers, Labrador retrievers, beagles, and schnauzers all have been reported to be predisposed to these tumors.[1,5,8,9] Although boxers are at increased risk of developing MCTs (accounting for nearly half of the dogs in one large series), they more commonly develop histologically low or intermediate grade forms of the disease, which have a more favorable prognosis.[1] No gender predilection has been reported.

Multiple, spontaneously regressing, cutaneous MCTs in a 3-week-old Jack Russell terrier have been described.[7] All the tumors regressed within 27 weeks. This syndrome of spontaneous regression in the young implies a hyperplastic or dysplastic syndrome rather than a true neoplastic lesion.

The etiology of MCTs in the dog is largely unknown. On rare occasions MCTs have been associated with chronic inflammation or the application of skin irritants.[10-12] Unequivocal evidence is lacking for a viral etiology, although MCTs have been transplanted to very young or immunocompromised laboratory dogs through tumor cell tissues and, rarely, cell-free extracts.[13-15] No C-type or other identifiable viral particles have been observed, and no epidemiologic evidence suggests horizontal transmission. Chromosomal fragile site expression, a phenomenon thought to genetically predispose humans to the development of certain tumors, was shown to be increased in boxer dogs with MCTs.[16] However, the control population for this study comprised young, non-tumor-bearing boxers, and the increased expression of chromosomal fragile sites likely was due to this age difference.

The genetic alterations that predispose an animal to canine MCTs are incompletely understood. Alterations in the p53 tumor suppressor pathway have been identified in some canine MCTs,[17-19] but p53 sequencing in a limited number of cases has revealed no mutations.[20] Perturbations in expression of the proteins p21 and p27, cyclin-dependent kinase inhibitors that contribute to regulation of the cell cycle, have been identified in many canine MCTs.[21] Recently, expression of c-kit, a tyrosine kinase receptor for the hematopoietic growth factor stem cell factor (SCF), has been demonstrated in canine and feline MCTs.[22-25] In addition to supporting the growth of normal mast cells in culture, SCF has been shown to induce secretion of mast cell granule constituents *in vivo* and *in vitro*.[26] Several studies have demonstrated, in a significant proportion of canine MCTs, the presence of mutations in the juxtamembrane region of c-kit, leading to constitutive activation in the absence of bound SCF.[27-31] This phenomenon appears to be more common in intermediate and high grade canine MCTs[28] and may be associated with a poorer prognosis.[30]

Cytosolic receptors for estrogen and progesterone also have been detected in canine MCTs.[32] Their role in the etiopathogenesis of MCTs is poorly understood, but evidence exists that estrogen and progesterone may influence mast cell function.[33] One European study reported that female dogs with MCTs had a more favorable prognosis with chemotherapy.[34] Although most studies performed in the United States have failed to detect such an association, the relatively higher

TABLE 19-1 Histologic Classification of Mast Cell Tumors in Dogs

Grade	Patnaik Grading	Microscopic Description
Anaplastic, undifferentiated (high grade)	3	Highly cellular, undifferentiated cytoplasmic boundaries; nuclei irregular in size and shape; frequent mitoses; sparse cytoplasmic granules
Intermediate grade	2	Cells closely packed with indistinct cytoplasmic boundaries; nucleus-to-cytoplasm ratio lower than in anaplastic type; infrequent mitoses; more granules than in anaplastic type
Well differentiated (low grade)	1	Clearly defined cytoplasmic boundaries with regular, spherical, or ovoid nuclei; rare or absent mitoses; abundant, large, deep-staining cytoplasmic granules

TABLE 19-2 Relative Frequency of Canine Mast Cell Tumors by Histologic Grade

Investigator	Number of Dogs	High (%)	Intermediate (%)	Low (%)
Hottendorf[49]	300	19	27	54
Bostock[42]	114	39	26	34
Patnaik[8]	83	20	43	36
Simoes[63]	87	22	40	38

frequency of unspayed females present in the European population may have allowed the effect of sex hormones to have a greater statistical impact on biologic behavior.

Pathology and Natural History

Cutaneous MCTs are thought to arise from tissue mast cells in the dermis and subcutaneous tissues.[2] Well-differentiated mast cells contain cytoplasmic granules that become larger as the cell matures. These granules contain a large number of bioactive constituents, including histamine and heparin, which stain metachromatically with toluidine blue.

Wide variation is seen in the histologic pattern of canine MCT, and the histologic grade has been clearly established as a strong prognostic factor that is highly predictive of biologic behavior and clinical outcome. Several investigators have applied histologic grading systems to canine mast cell tumors based on the degree of differentiation (Table 19-1). The number grades used in these studies are at odds; therefore, for the sake of clarity, the three differentiation groups should be referred to simply as undifferentiated (high) grade, intermediate grade, and well-differentiated (low) grade. Table 19-2 lists the relative distribution of MCT grades in larger series. Highly anaplastic, agranular MCTs may

be difficult to diagnose by routine light microscopy. Immunohistochemical techniques have been applied in an attempt to differentiate these tumors from other anaplastic round cell tumors.[19,22,24] Mast cell tumors are vimentin positive, and most are alpha-1-antitrypsin positive. As stated previously, most also stain positively for CD117 (kit). In highly anaplastic tumors, the electron microscopy ultrastructural appearance may be required for definitive confirmation.

The true metastatic potential of canine MCT is not known for certain. The histologic grade appears to be very predictive of metastatic behavior. Early necropsy reports state that the metastatic rate of MCT can be as high as 96%.[35] However, this is an overestimation, because the necropsy subjects in this generally untreated series either died or were euthanized as a direct result of their tumors; therefore most of the dogs in the series probably had anaplastic, undifferentiated tumors, which have a worse prognosis. The clinical experience of the authors and others suggests that the metastatic potential of well-differentiated tumors is low (less than 10%) and that of intermediate grades is low to moderate.[36-41] The metastatic rate for undifferentiated tumors ranges from 55% to 96%.[42] Most of these tumors disseminate first to the local lymph nodes and then to the spleen and liver. Other visceral organs may be involved,

although lung involvement is uncommon. Neoplastic mast cells may be observed in the bone marrow and peripheral blood in cases of widespread systemic dissemination. Bone marrow involvement occurs in as many as half of the cases of anaplastic visceral MCT.[43]

Complications related to the release of the bioactive constituents of mast cell granules can occur with MCTs. Gastrointestinal ulceration is common, reported to occur in 35% to 83% of necropsy specimens (Figure 19-1).[44,45] However, the fact that these numbers arose from a necropsy population likely overstates the prevalence of ulceration. A small percentage of these cases may go on to perforate. Histamine released from MCT granules is thought to act on parietal cells via H_2 receptors, resulting in increased secretion of hydrochloric acid. Plasma histamine concentrations are increased in dogs with MCTs,[46] and preliminary data indicate that monitoring of plasma histamine concentrations may be useful for monitoring disease progression.[46] These dogs also have decreased concentrations of plasma gastrin, which normally is released by antral G-cells in response to an increased concentration of gastric hydrochloric acid, acting as a negative feedback loop. Increased gastric acid secretion combined with vascular damage likely is the cause of gastric ulceration.[44] Perioperative degranulation of MCTs and subsequent release of histamine and other, less characterized vasoactive substances may also
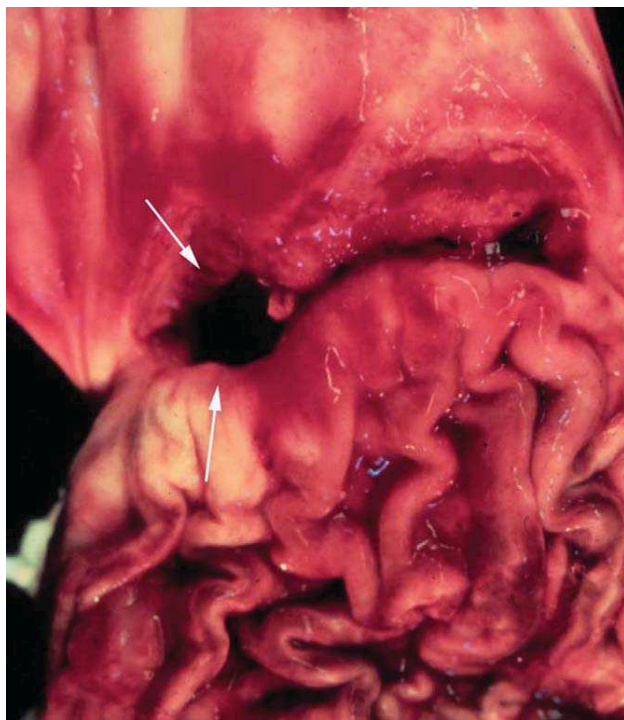
result in potentially life-threatening hypotensive events during surgery. Some researchers believe that prostaglandins in the D series secreted by tumor cells may mediate the hypotensive effects observed in humans with mast cell diseases.[47,48] Coagulation abnormalities, also reported in dogs with MCTs, likely are caused by heparin release from mast cell granules.[43,49] Clinical evidence of hemorrhage is not typically associated with this phenomenon; however, localized hemorrhage at the time of surgery, caused by degranulation resulting from tumor manipulation, can be a serious complication, even if presurgical coagulation parameters are normal.

Delayed wound healing at the site of removal of a MCT has been attributed to the local effects of proteolytic enzymes and vasoactive amines released by the tumor. Studies in mice suggest that histamine released from the tumor binds to H_1 and H_2 receptors on macrophages, resulting in release of a fibroblastic suppressor factor that decreases normal fibroplasia and delays wound healing.[50] Also, histamine and human mast cell leukemia lysates have been shown to reduce keratinocyte proliferation, further inhibiting wound epithelialization.[51]

History and Clinical Signs

Cutaneous MCTs have an extremely varied range of clinical appearances. Most tumors are solitary, but 11% to 14% of animals have multiple lesions.[38,52,53] The appearance of these tumors has been correlated with the degree of histologic differentiation.[42] Well-differentiated MCTs tend to be solitary, 1 to 4 cm in diameter, slow growing, and rubbery, and they often are present for at least 6 months. They are not typically ulcerated, but the overlying hair may be lost (Figure 19-2). Undifferentiated MCTs tend to be rapidly growing, ulcerated lesions that cause considerable irritation and attain a large size (Figure 19-3). Surrounding tissues may become inflamed and edematous, and small satellite nodules may develop in the surrounding tissues. Tumors of intermediate differentiation fill the spectrum between these two extremes. A subcutaneous form of MCT that is soft and fleshy on palpation often is misdiagnosed clinically as a lipoma (Figure 19-4).

The history and clinical signs in dogs with an MCT may be complicated by signs attributable to the release of histamine, heparin, and other vasoactive amines. Occasionally, mechanical manipulation during examination of the tumor results in degranulation and subsequent erythema and wheal formation in surrounding tissues, a phenomenon referred to as *Darier's sign* (Figure 19-5).[52] As previously discussed, gastrointestinal ulceration may occur, producing related signs of vomiting (possibly with blood), anorexia, melena, and abdominal pain.

Figure 19-1

Perforated ulcer in the pylorus of a dog with an extensive mast cell tumor.

Figure 19-2

Well-differentiated mast cell tumor on the lateral aspect of the antebrachium. The overlying hair has been lost. This tumor had been present for 3 months before presentation.

Figure 19-4

Cross section of a subcutaneous mast cell tumor from the shoulder of a dog. This mass originally was misdiagnosed as a lipoma based on palpation alone. Note the tumor extension through the fascia into the muscle. Wide surgical excision that includes the deep muscle layer is necessary to achieve complete (clean) surgical margins.

Figure 19-3

A large, rapidly growing, undifferentiated mast cell tumor on the hip of a 9-year-old black Labrador retriever. The tumor has ruptured, likely because the rapid growth outstripped the available blood supply, resulting in necrosis.

Figure 19-5

Erythema and wheals developed in the surrounding skin after manipulation of this cutaneous mast cell tumor; this phenomenon, known as Darier's sign, is caused by the release of vasoactive amines from mast cell granules.

Normal mast cells are found in abundance in the lung and gastrointestinal tract, but these sites show low prevalence rates for the development of MCTs. In the dog, cutaneous MCTs most often arise from the dermis and subcutaneous tissues. They most commonly are found on the trunk (approximately 50% to 60% of sites).[5,42] The limbs account for approximately one fourth of the sites of these tumors. Lesions are least common on the head and neck. Mast cell tumors have been reported to occur infrequently in other sites, including the conjunctiva, salivary gland, nasopharynx, larynx, oral cavity, gastrointestinal tract, ureter, and spine.[54-56]

A visceral form of MCT, often referred to as *disseminated* or *systemic mastocytosis*, also can occur.[43,57-60] In the dog, visceral MCT is almost always preceded by an undifferentiated primary cutaneous lesion. Consistent abnormalities include lymphadenopathy, splenomegaly, and hepatomegaly, representing disseminated MCT. Bone marrow and peripheral blood involvement with neoplastic mast cells are common. Pleural and peritoneal effusions containing abundant neoplastic mast cells have been observed in a number of dogs with visceral MCT.

A case series of dogs with primary gastrointestinal MCT recently was reported.[19] The tumors were well distributed throughout the gastrointestinal tract, and most of the dogs were presented for treatment of vomiting, diarrhea, and melena. Only 40% of the dogs were alive 30 days after first admission, and fewer than 10% were alive at 6 months.

Prognostic Factors

A discussion of prognostic factors associated with canine MCT precedes the sections on diagnosis and treatment, because the steps followed in those sections are predicated on the presence or absence of these prognostic factors. Table 19-3 lists factors known to be predictive of the biologic behavior of the tumor and the clinical outcome in dogs with MCT.

The histologic grade is the most consistent prognostic factor available for dogs with MCT.[61] The survival

TABLE 19-3	Prognostic Factors for Mast Cell Tumors in Dogs
Factor	**Comment**
Histologic grade	Strongly predictive of outcome; dogs with undifferentiated tumors typically die of their disease after local therapy alone, whereas dogs with intermediate grade or well-differentiated tumors usually are cured with appropriate local therapy.[8,35,42,61,63]
Clinical stage	Stage 0 and stage I tumors, confined to the skin without local lymph node or distant metastasis, have a better prognosis than higher stage disease.[34,42,67,71]
Location	Preputial, scrotal, subungual, oral, and other mucous membrane sites are associated with more high grade tumors and a worse prognosis. Visceral or bone marrow disease usually carries a grave prognosis.[68-72]
Cell proliferation rate	The mitotic index, the relative frequency of argyrophilic nucleolar organizer regions (AgNORs), and the percentage of proliferating cell nuclear antigens (PCNAs) or Ki-67 immunopositivity are predictive of the postsurgical outcome.[62-66]
Growth rate	Mast cell tumors (MCTs) that remain localized and are present for prolonged periods (months or years) without significant change usually are benign.[42]
DNA ploidy	Dogs with aneuploid tumors tend to have shorter survival times and higher stage disease.[67]
Microvessel density	Increased microvessel density is associated with a higher tumor grade, a higher degree of invasiveness, and a worse prognosis.[65]
Recurrence	Local recurrence after surgical excision may have a more guarded prognosis.[73]
Systemic signs	Systemic illness (e.g., anorexia, vomiting, melena, gastrointestinal ulceration) may be associated with a higher stage of disease.[38,60,69]
Age	Older dogs may have shorter median disease-free intervals when treated with radiotherapy.[89]
Breed	MCTs in boxers (and possibly other brachycephalic breeds) tend to be low or intermediate grade and therefore are associated with a better prognosis.[1]
Gender	In one study, male dogs had a shorter survival time than female dogs when treated with chemotherapy.[35]
Tumor size	Large tumors may be associated with a worse prognosis after surgical removal and/or radiotherapy.[38]
c-Kit mutation	A juxtamembrane region mutation of c-Kit may be associated with a worse prognosis.[28,30]

time after surgical excision, based on tumor grade, is presented in Table 19-4. Most dogs with well-differentiated tumors (80% to 90%) and approximately 75% of dogs with moderately differentiated tumors experience a long-term survival after complete surgical excision. Dogs with undifferentiated tumors treated surgically typically die of their disease within 1 year as a result of local recurrence or metastasis. Recently, silver colloid staining of paraffin-embedded sections to determine the relative presence of so-called argyrophilic nucleolar organizer regions (AgNORs) has been correlated with the histologic grade and postsurgical outcome.[62,63] AgNORs are thought to be an indirect measure of cell proliferation. In a study of 50 dogs with cutaneous MCT, the AgNOR frequency was as predictive or even more predictive of biologic behavior than the histologic grade.[62] This relatively simple procedure can be performed on both cytologic and histologic specimens, and in the future it may provide simple, readily

available prognostic information.[64] Assessment of the mitotic index and immunohistochemical assessment of proliferating cell nuclear antigen (PCNA) or Ki-67 immunoreactivity, other indirect measurements of tumor proliferation, also have been shown to correlate with the postsurgical outcome.[63,65,66] A study of deoxyribonucleic acid (DNA) ploidy determined by flow cytometric analysis suggested a trend toward a shorter survival time and a higher clinical stage of disease in aneuploid tumors compared with diploid tumors.[67] However, simple light microscopy criteria have greater predictive value, therefore ploidy assessment is not a cost-effective procedure for MCT. A recent study found a strong correlation linking the intratumor microvessel density and invasiveness, the mitotic rate, and the prognosis.[65]

Several investigators hold that tumor location can be used to predict outcome.[68-71] Tumors in the preputial/inguinal area (Figure 19-6), subungual (nail bed) region

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TABLE 19-4   Survival Times of Dogs with Surgically Treated Mast Cell Tumors According to Histologic Grade*

Investigator/Tumor Grade	Number of Dogs	Percentage Alive/Months After Surgery	Median Survival Time (Weeks)
Bostock[42]			
Low grade	39	79/7	Not reported (NR)
Intermediate grade	30	37/7	
High grade	45	15/7	
Patnaik[8]			
Low grade	30	83/48	NR
Intermediate grade	36	44/48	
High grade	17	6/48	
Bostock[62]			
Low grade	19	90/NR	>40†
Intermediate grade	16	75/NR	>36†
High grade	15	27/NR	13
Murphy[39]			
Low grade	87	100/12	>80†
Intermediate grade	199	92/12	>80†
High grade	54	46/12	40
Simoes[63]			
Low grade	33	91/20	NR
Intermediate grade	35	71/20	
High grade	19	42/20	

*These studies are unclear on whether death was caused by metastasis or by local recurrence; the completeness of local resection was rarely reported.
†Medians not reached at the time of last follow-up (i.e., >50% alive).

Figure 19-6

Mast cell tumors in the preputial area are nearly always anaplastic tumors, as was the case in this retriever. Tumors in this area have a high metastatic potential, spreading to inguinal lymph nodes and beyond. Note the edema and Darier's sign in the surrounding tissues.

(Figure 19-7), and other mucocutaneous sites, including the oral cavity and perineum, are anecdotally associated with aggressive behavior. However, two recent case series suggested no difference in outcome for dogs with inguinal or perineal MCTs compared to dogs with tumors in other cutaneous sites.[36,72] These studies did not specify the percentage of dogs with tumors arising from haired skin rather than mucous membranes in the perineum.[36] In addition, although no *statistical* difference was noted, the median disease-free interval (DFI) for dogs with MCTs in the inguinal or perineal region was 9.6 months, compared with 33.9 months for dogs with tumors in other cutaneous sites. Also, when dogs with preputial and scrotal MCTs were specifically separated out, a statistically significant difference in DFI emerged (median of 4.2 months compared with 33.9 months).[72] Based on these data, dogs with MCTs at these sites still should be considered at increased risk for recurrence or metastasis. One series of MCTs treated with radiotherapy after incomplete surgical excision reported a significant survival advantage with tumors on the extremities compared with those on the trunk.[71] This may reflect the poor prognosis associated with tumors in inguinal and perineal sites. Another recent study demonstrated that a mucosal location was associated with a worse prognosis in dogs with MCT and a high risk for metastasis that were treated with adjuvant chemotherapy.[61]

The clinical stage (Table 19-5) also is predictive of outcome.[34,42,67,71] However, several studies have suggested that no difference in outcome exists between patients with a single MCT and those with multiple cutaneous MCTs.[38,61,73] Therefore this part of the staging scheme may not accurately correlate with outcome. Bostock[42] reported that the growth rate, which is determined by
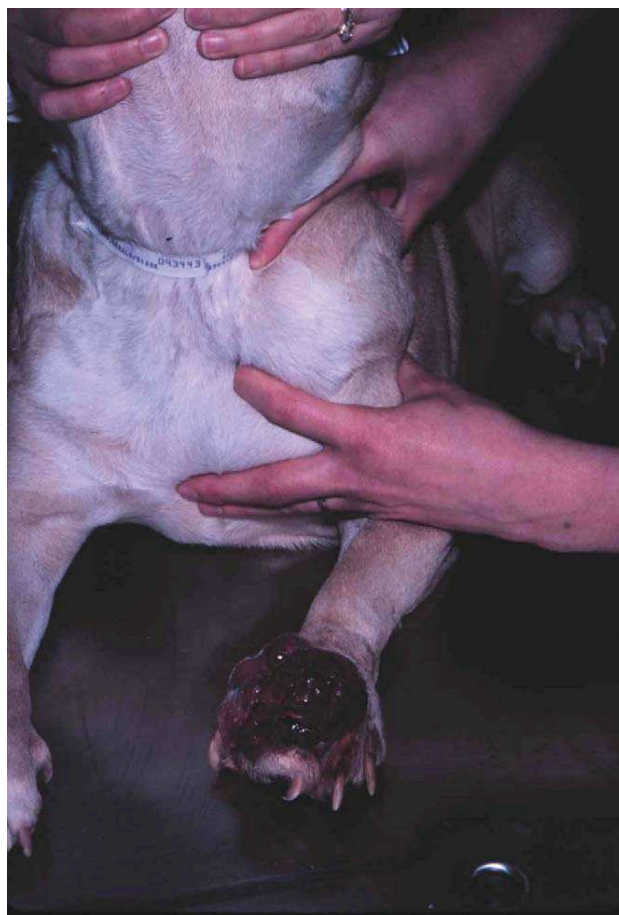
Figure 19-7

Subungual undifferentiated mast cell tumor in an English bulldog. As with most mast cell tumors in this location, lymph node metastasis has occurred.

dividing the tumor volume by the time in weeks that the tumor is present, was significantly prognostic. More simply stated, recent rapid growth is a negative prognostic sign, whereas tumors present longer than 26 weeks have a good prognosis.

Systemic signs of anorexia, vomiting, melena, widespread erythema, and edema associated with vasoactive substances from mast cell degranulation are more commonly associated with visceral forms of MCT and as such warrant a more guarded prognosis.[38,60,69] In 16 cases of visceral MCT, a median survival of 90 days was reported, and all dogs with follow-up died of their disease.[69] Local tumor ulceration, erythema, or pruritus also has been associated with a worse prognosis in some studies.[38,73] Recurrence of MCTs after surgical excision has been associated with a more guarded prognosis (Figure 19-8).[73] Therefore appropriately aggressive therapy at the time of first presentation, rather than at the time of recurrence, may improve the long-term prognosis in patients with MCT.

TABLE 19-5	World Health Organization Clinical Staging System for Mast Cell Tumors
Stage	**Description**
0	One tumor incompletely excised from the dermis, identified histologically, without regional lymph node involvement
	0a: Without systemic signs
	0b: With systemic signs
I	One tumor confined to the dermis, without regional lymph node involvement
	Ia: Without systemic signs
	Ib: With systemic signs
II	One tumor confined to the dermis, with regional lymph node involvement
	IIa: Without systemic signs
	IIb: With systemic signs
III	Multiple dermal tumors*; large, infiltrating tumors with or without regional lymph node involvement
	IIIa: Without systemic signs
	IIIb: With systemic signs
IV	Any tumor with distant metastasis, including blood or bone marrow involvement

Multiple tumors have not been documented to have a worse prognosis.

Figure 19-8

Local recurrence of a mast cell tumor on the antebrachium within 1 month of attempted excision. Recurrence often is associated with more aggressive tumors.

Diagnostic Technique and Workup

Mast cell tumors initially are diagnosed on the basis of fine-needle aspiration (FNA) cytology. The Romanowsky or rapid hematologic–type stains used in most practices suffice. Mast cells appear as small to medium-sized round cells with abundant, small, uniform cytoplasmic granules that stain purplish red (metachromatic) (Figure 19-9).[1,74] A small percentage of MCTs have granules that do not stain readily, giving them an epithelial or macrophage-like appearance often referred to as a "fried egg" appearance (Figure 19-10). In these cases a Wright-Giemsa stain often reveals granules, although histologic assessment ultimately may be necessary for diagnosis. Although FNA is convenient for diagnosis, the clinician must keep in mind that it does not provide a histologic grade.

The extent of ancillary diagnostic workup after FNA cytologic diagnosis is predicated on the presence or absence of the negative prognostic factors discussed previously. Figure 19-11 shows the diagnostic steps and the order in which they generally are pursued in the authors' practice. If the MCT is in a location amenable to wide surgical excision and no negative prognostic indicators are present (see Table 19-3), no further diagnostic staging is done at this point, and wide surgical excision is performed. The excised tissue is submitted *in toto* for assessment of histologic grade and completeness of surgical removal (margins). FNA cytology is not sufficient for grading a MCT, therefore histologic assessment is strongly recommended. Further diagnostics and therapeutics may be performed if the excised specimen is
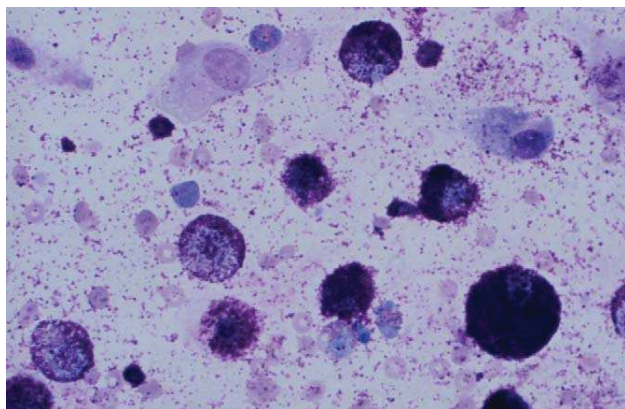
Figure 19-9

Fine-needle aspirate of a mast cell tumor in a dog. Note the individual round cells with round to oval nuclei that are obscured by an abundance of fine, basophilic, cytoplasmic granules. Numerous mast cell granules are present in the background. (Wright's stain, 1000×.) *(Photomicrograph courtesy Dr. Karen Young, University of Wisconsin–Madison.)*
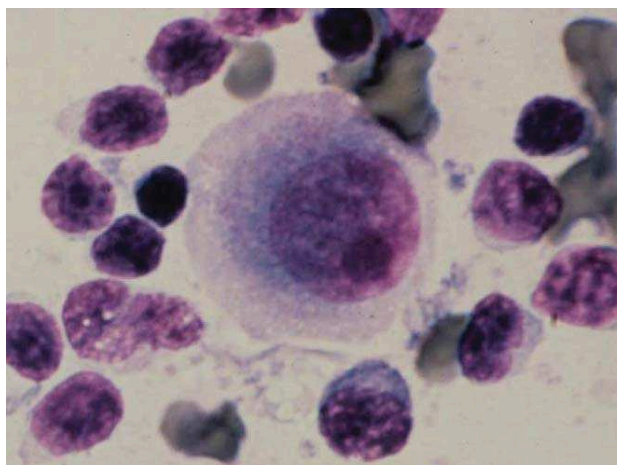
Figure 19-10

Fine-needle aspirate of a mast cell tumor in a dog. Note the absence of granules in the large tumor cell. On a Wright's-stained specimen from the same tumor, granules were evident in the tumor cells. (Diff-Quik stain, 1000×.) *(Photomicrograph courtesy Dr. Karen Young, University of Wisconsin–Madison.)*
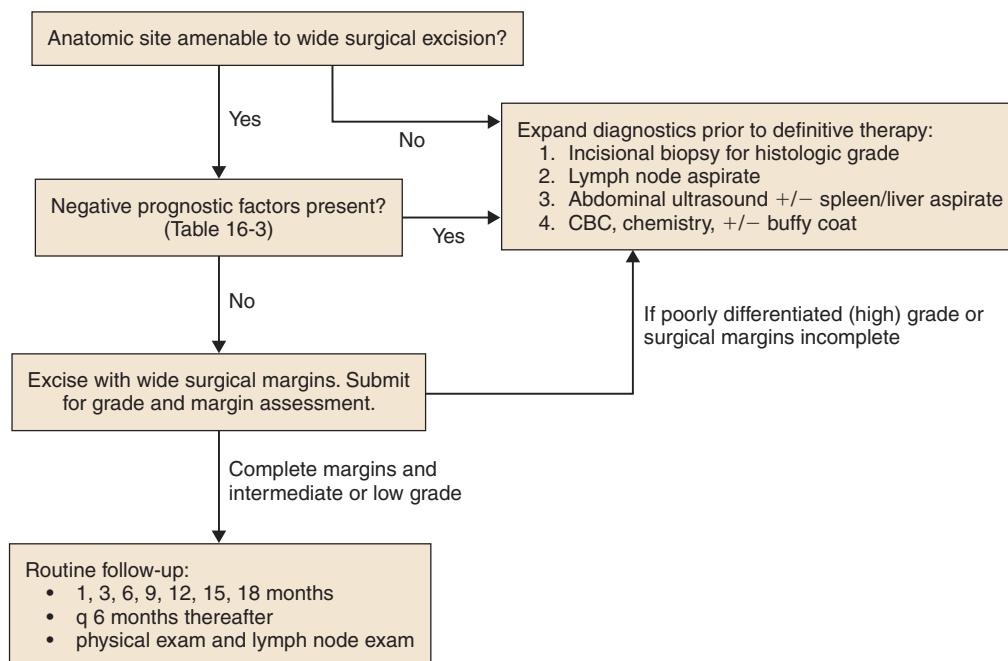
Figure 19-11

Suggested diagnostic steps for canine cutaneous mast cell tumors.

determined to be an undifferentiated tumor or if surgical margins are incomplete, requiring more aggressive or costly definitive treatment of the primary site.

If the tumor develops at a site that is not amenable to wide surgical excision (e.g., a distal extremity) or if negative prognostic factors exist in the history or physical examination, ancillary diagnostics to further stage the disease are recommended before definitive therapy. Historically, complete staging has included cytologic assessment of regional lymph nodes, a complete blood

count (CBC) and buffy coat smear to document peripheral mastocytosis, abdominal ultrasound with cytologic assessment of the spleen or liver if warranted, thoracic radiographs, and bone marrow aspiration. An incisional biopsy often is performed at this point to determine the histologic grade. The results of the more extensive workup allow the clinician to better inform clients about the probable outcome after treatment. They also aid the planning of the type and extent of definitive therapy.

Opinion recently has shifted on the issue of the extent of staging required for most dogs with MCTs. The minimum advisable in cases requiring presurgical staging consists of standard geriatric preanesthetic blood work, needle aspiration cytology of the regional node (even if normal in size), and abdominal ultrasound. Thoracic radiographs rarely demonstrate metastasis; however, they are very reasonable as an "insurance policy" before an expensive or invasive procedure to rule out unrelated disease processes or occult cardiopulmonary disease that could complicate anesthesia. Aspiration cytology of a structurally normal liver or spleen generally is unrewarding, and interpretation can be complicated by the presence of nonmalignant mast cells in these organs in normal dogs.

In the visceral form of canine MCT, buffy coat smears are positive for mast cells in 37% of cases, and 56% of bone marrow aspirates reveal mast cell dissemination.[69] However, these cases constitute a small minority of all MCT cases, and the presence or absence of disease in blood or marrow rarely alters treatment decisions, because visceral MCT is a uniformly fatal disease. With the exception of the extremely rare case of primary mastocytic leukemia,[75,76] involvement of marrow or peripheral blood is unlikely in the absence of disease in the regional lymph nodes or abdominal organs.

When ancillary tests for staging are performed, the clinician must keep in mind that mast cells are also found in normal tissues. In 56 healthy beagles, approximately 24% of lymph node aspirates contained mast cells (range, 1 to 16 mast cells per slide; mean, 6.4 mast cells per slide).[77] With regard to lymph node cytology, an occasional solitary mast cell is not indicative of metastasis; clustering and aggregates are more worrisome (Figure 19-12). Surgical extirpation of a cytologically suspicious node for histologic assessment is recommended. Of 51 bone marrow samples examined, two slides contained a single mast cell. No mast cells were observed in any of the 53 buffy coat smears examined from these dogs.[78-80] However, peripheral mastocytosis (1 to 90 mast cells/μl) is reported in dogs with acute inflammatory disease (particularly parvoviral infections), inflammatory skin disease, regenerative anemias, neoplasia other than MCT, and trauma.[78-80] One study revealed that peripheral mastocytosis actually is more likely to occur and may be more dramatic in dogs with diseases other than MCT.[79] Therefore the dogma that buffy coat smears are an important diagnostic assessment
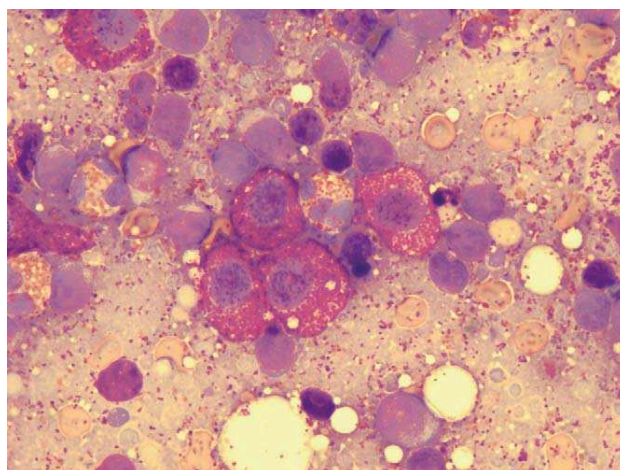
Figure 19-12

Regional lymph node aspirate from a dog with a cutaneous mast cell tumor. Note the clustering of mast cells in a background of lymphocytes; this is more indicative of true metastasis.

of MCT patients should be tempered and may indeed be of more benefit if used to assess changes over time.

To summarize, in the authors' practice, if staging is indicated, we limit diagnostics to regional node assessment, abdominal ultrasound with aspiration of "abnormal" organs, and baseline thoracic radiographs. Buffy coat smears, bone marrow aspiration, and aspiration of structurally normal abdominal organs generally is not indicated.

Knowledge of the extent of gross MCT margins before surgery, usually acquired by digital palpation and occasionally by local radiographs, can be enhanced by using diagnostic ultrasound (US) or computed tomography (CT). In one study involving dogs with cutaneous mast cell tumors and soft tissue sarcomas, the extent of local tumor margins was upgraded in 19% of cases with US and in 65% of cases with CT.[81] Such information allows more appropriate planning of definitive surgery or radiotherapy. The cost-effectiveness of such studies depends on the location of the tumor and whether wide excision is technically simple or difficult.

Treatment

Treatment decisions are based on the presence or absence of negative prognostic factors and on the clinical stage of disease. Surgical excision and radiation therapy are the most successful treatment options described to date. In tumors localized to the skin in areas amenable to wide excision, surgery is the treatment of choice. Historically, surgical excision that includes a 3 cm margin of surrounding normal tissue has been recommended for MCTs (Figure 19-13, *A*). More recently, evidence has indicated that 2 cm lateral margins may be sufficient for complete excision of most MCTs.[82] Extensive deep

A   B
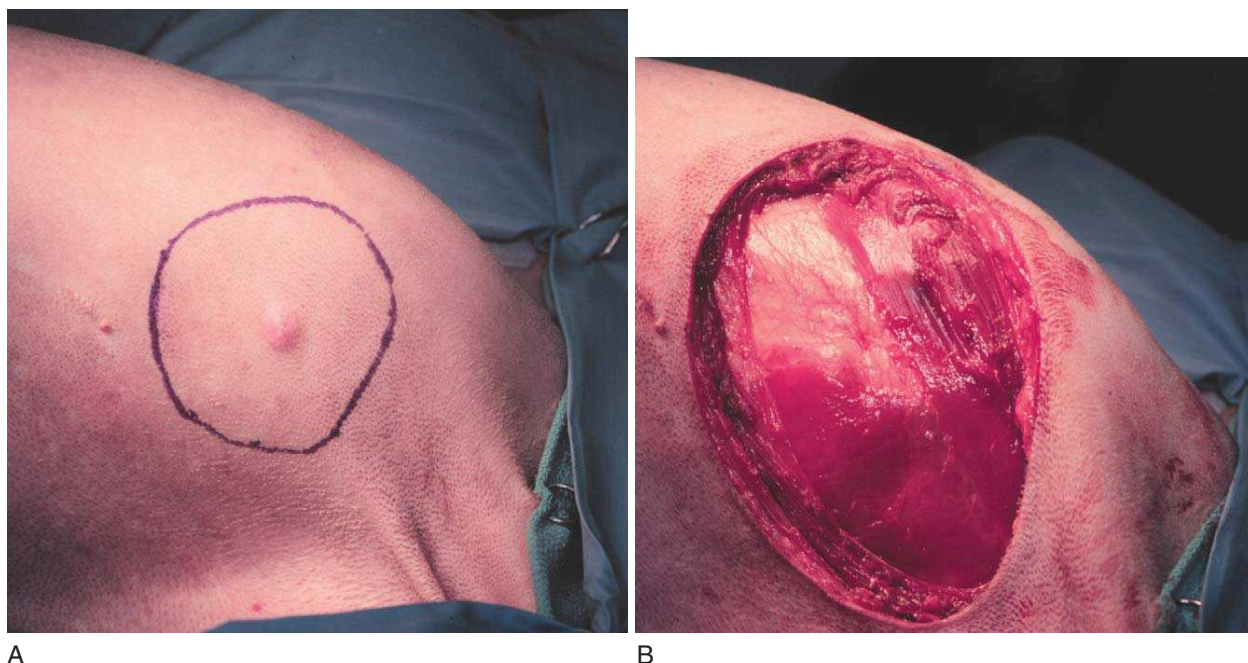
Figure 19-13

Surgical excision of a cutaneous mast cell tumor from a dog. **A,** The planned wide surgical margins. **B,** Note the deep fascial layer that was removed to achieve complete excision. *(Courtesy J. McAnulty, University of Wisconsin–Madison.)*

margins are as important as lateral margins, and one fascial plane deep to the tumor should be removed in continuity with the tumor (Figure 19-13, *B*). If necessary, muscle layers may also be removed deep to the tumor. All surgical margins should be evaluated histologically for completeness of excision. Tumors in areas not amenable to such wide surgical margins (e.g., the distal extremities) should be evaluated by incisional biopsy for histologic grade before definitive therapy.

If a distal extremity MCT is low or intermediate grade, three primary therapeutic options exist. The most aggressive option is amputation; however, this is probably too much surgery for most low and intermediate grade tumors. Amputation generally guarantees wide margins, but it results in the least functional outcome. The second option is external beam radiotherapy alone, which, in the literature, has produced varying control rates when used as a primary therapy.[71,83-87] Radiotherapy alone for measurable tumors at dosages of 40 to 45 Gray results in a 1-year control rate of approximately 50%. However, the more aggressive total doses of 48 to 57 Gray now used by many veterinary radiotherapy facilities may improve these results, and the addition of chemotherapy to radiotherapy may improve the response rate and duration.

The third, and in the authors' opinion, the ideal option for low or intermediate grade MCTs in areas where wide surgical excision is not possible is a combination of surgery and radiotherapy. The veterinary literature has established that complementary use of surgery to achieve clinical stage 0 disease (i.e., microscopically incomplete margins) followed by external beam radiotherapy is associated with long-term control in most cases. A 2-year control rate of 85% to 95% can be expected for stage 0 low or intermediate grade tumors.[71,88-90] Many authors advocate prophylactic nodal irradiation (PNI) of cytologically negative regional lymph nodes.[61,90-92] Because of the generally low risk of metastasis in low to intermediate grade tumors,[40] this procedure probably is unwarranted in this group of patients, and at least one study has demonstrated no advantage in terms of disease-free or overall survival as a function of PNI in such patients.[93] However, with MCTs at high risk for metastasis, PNI may result in an improved outcome compared with local site irradiation only.[61] Regardless of the local therapy chosen, dogs with low and intermediate grade tumors should be re-evaluated regularly for local recurrence and possible systemic spread. In our practice, we re-evaluate patients 1 month after definitive therapy, then every 3 months for $1\frac{1}{2}$ years, and then every 6 months thereafter. Local site and regional lymph node evaluation, a complete physical examination, and aspiration of any new cutaneous masses are performed at these intervals.

Figure 19-14

Re-excision of a mast cell tumor from the skin of a golden retriever. The first surgery resulted in incomplete surgical margins. In the re-excision procedure, 3 cm margins were taken around and deep to the previous incision, and the entire sample was again submitted for pathologic examination for residual tumor and margin analysis.

More complete staging, including abdominal US, is pursued if warranted by these findings.

When planned curative excisional surgery is unsuccessful and histologic margins are reported as incomplete, further local therapy generally is warranted. A second excision with additional wide margins of the surgical scar, if possible (Figure 19-14), or adjuvant radiotherapy is recommended. Adjuvant chemotherapy also is an option in this setting (see discussion later in the chapter); however, failure is more likely to occur locally in low to intermediate grade MCTs, and chemotherapy is a "systemic" approach. Not all MCTs with surgically incomplete margins recur; in one report, only 30% of MCTs with histologically confirmed incomplete margins did so.[94] Although recurrence rates vary by study, several studies have demonstrated increased recurrence rates or decreased overall survival (or both) in dogs with incompletely resected MCTs.[37-39]

Figure 19-15 summarizes the treatment recommendations for clinical stage 0 and stage I, histologically low or intermediate grade MCTs. Alternative local therapies for confined MCTs that have been reported include hyperthermia with radiotherapy,[95] intralesional brachytherapy,[96,97] photodynamic therapy,[98,99] intralesional corticosteroids,[100] cryotherapy, and intralesional deionized water.[101-105] None have been as thoroughly investigated or are as clinically effective or practical as surgery or radiation therapy or a combination of these two. Even though additional adjuvant corticosteroid therapy is commonly used, no clinical data suggest that it has any benefit in cases of intermediate grade MCTs that were excised completely or treated with local radiotherapy.

The treatment of anaplastic or undifferentiated MCT remains a frustrating undertaking for both the client and the clinician. Historically, this designation includes dogs with intermediate grade tumors with regional or distant spread. Some evidence suggests that intermediate grade tumors with only regional node involvement have a better prognosis than high grade tumors.[61] In the authors' opinion, until convincing evidence exists, such tumors should be treated as though they were high grade lesions. Client education with respect to the prognosis is essential in the decision-making process. We treat undifferentiated tumors with aggressive local surgery or radiotherapy only if thorough staging fails to reveal dissemination and if the client has been fully informed as to the likelihood of future dissemination. Figure 19-16 summarizes the treatment recommendations for high grade MCTs. The long-term prognosis for such dogs is less favorable because regional and distant metastasis is more likely, especially in high-risk locations such as the preputial and perianal areas. Because the prognosis is guarded for high grade tumors, the option of a less expensive, coarse fraction radiation therapy protocol (e.g., 4 weekly, 8 Gray fractions) can be considered as an alternative to more traditional fractionation schemes when radiotherapy is contemplated in this setting.

In most instances, poorly differentiated and metastatic MCT kills the host in the absence of effective postsurgical intervention. Systemic adjuvant therapy should be offered in such cases to try to reduce the likelihood of systemic involvement or at least possibly to improve the disease-free interval. For many years, the use of corticosteroids (e.g., prednisone) has been reported to be of some benefit in preclinical and anecdotal settings.[106-108] Glucocorticoid receptors have been identified in the cytosol of canine MCT cells, and a recent study demonstrated inhibition of canine MCT proliferation and probable induction of tumor cell apoptosis by glucocorticoids both *in vitro* and in a murine xenograft.[109] Glucocorticoids also may contribute to the apparent antitumor response by reducing peritumoral edema and inflammation. The Veterinary Cooperative Oncology

Anatomic site amenable to wide surgical excision?

Yes → **Excise with wide surgical margins. Analyze margins and confirm grade.**

No → **Options:**
Ideal: cytoreductive surgery and adjuvant radiotherapy
Alternative choices:
1. Amputation if on limb
2. Cytoreduce and follow with vinblastine/prednisone
3. Radiotherapy alone (possible surgery to follow)
4. Vinblastine/prednisone alone (possible surgery to follow)

Complete surgical margins?

No → **Options:**
1. Reexcision with wider margins (submit for completeness)
2. Adjuvant radiotherapy
3. Adjuvant chemotherapy (vinblastine/pred)

Yes → **Routine follow-up:**
- 1, 3, 6, 9, 12, 15, 18 months
- q 6 months thereafter
- physical exam and lymph node exam

Figure 19-15

Suggested treatment approach for clinical stage 0 and stage I, low or intermediate grade canine mast cell tumors.

Anatomic site amenable to wide surgical excision?

Yes → **Excise with wide surgical margins. Analyze margins and confirm grade.**

No → **Options:**
Ideally a combination of:
- cytoreductive surgery
- adjuvant radiotherapy* including regional node
- adjuvant chemotherapy (vinblastine/prednisone)
Alternative choices:
1. Amputation if on limb and adjuvant chemotherapy
2. Cytoreduce and follow with vinblastine/prednisone
3. Vinblastine/prednisone alone
4. Radiotherapy* and vinblastine/prednisone
5. Radiotherapy alone*

Complete surgical margins?

No → **Options:**
1. Reexcision with wider margins
2. Adjuvant radiotherapy to primary site*

Yes → **Adjuvant chemotherapy (vinblastine/prednisone) Consider regional node irradiation***

*Note: Consider coarse fraction radiation therapy protocols in place of more traditional protocols due to poor prognosis with high grade tumors.

Routine follow-up:
- 1, 3, 6, 9, 12, 15, 18 months
- q 6 months thereafter
- physical exam and lymph node exam, abdominal ultrasound

Figure 19-16

Suggested treatment approach for high grade, biologically aggressive canine mast cell tumors.

Agent	Number Treated	Complete Response (%)	Partial Response (%)	Overall Response Rate (%)	Median Response Duration	Reference
Prednisone	25	4	16	20	Not reported	110
Vincristine	27	0	7	7	Not reported	112
CCNU (lomustine)	21	6	38	44	79 days*	113
Prednisone/vinblastine	17	33	13	47	154 days	73
P/C/V	21	0	78	78	Not reported	111
COP-HU	17	23	35	59	53 days	34

TABLE 19-6   Response to Chemotherapy in Canine Mast Cell Tumors

Excludes a patient that experienced a complete remission, which was euthanized without evidence of disease after 440 days.

P/C/V, Prednisone/cyclophosphamide/vinblastine; COP-HU, Cyclophosphamide/vincristine/prednisone/hydroxyurea.

Group (VCOG) has studied the efficacy of single agent systemic prednisone therapy for intermediate and high grade canine MCTs.[110] In 21 dogs that received 1 mg/kg given orally daily, only one complete response and four partial responses were noted. Responses were short-lived, lasting only a few weeks in most cases.

Recently, a number of studies have evaluated the response rate of measurable canine MCT to various systemic chemotherapeutic drugs and protocols (Table 19-6).[34,73,111-113] Response rates as high as 78% have been reported,[111] and preliminary evidence suggests that multiagent protocols may achieve a higher response rate than single agent therapy.[34,111]

A few studies have attempted to address the use of chemotherapy for MCTs after surgery. In one study, 27 dogs with incompletely or marginally resected MCTs, mostly of intermediate grade, were treated with prednisone and vinblastine (VBL) chemotherapy. Only one dog (3.7%) experienced local recurrence, and four (15%) developed a distant cutaneous MCT.[114] A second group of researchers evaluated the use of postoperative prednisone and VBL for dogs with MCTs considered to be at high risk for metastasis (node positive, mucous membrane origin, or high grade).[61] In this study, dogs with high grade MCTs had a median survival time of 1374 days. This is an apparent improvement over historical data on the use of surgery alone. In this adjuvant setting, VBL (2 mg/m^2 given intravenously) is administered weekly for four treatments, followed by four additional treatments given every 2 weeks. Prednisone is used concurrently with VBL at an initial dosage of 2 mg/kg given orally daily for 1 week, then 1 mg/kg given orally daily for 2 weeks, followed by 1 mg/kg given orally every other day. Prednisone is discontinued once VBL treatment is complete.

The issue of what should be done with intermediate grade MCTs after complete (i.e., clean) surgical excision continues to be a source of controversy. As previously stated, we now know that incompletely excised intermediate grade tumors can be effectively managed with adjuvant radiotherapy. However, the management of tumors excised with complete margins remains controversial. According to the findings of Bostock and colleagues[42,62] and Patnaik and coworkers,[8] 50% to 75% of the MCTs in this category are essentially cured. More recently, Seguin and colleagues[40] reported that 5% of completely excised, intermediate grade MCTs will recur, 5% will metastasize, and 11% of these patients may develop another, presumably unrelated cutaneous MCT in their lifetime.[40] Because of the apparent low likelihood of failure with complete excision, the authors' opinion is that no further therapy should be recommended; rather, these animals should be placed on a rigorous re-evaluation schedule (see Figure 19-14).

A syndrome of multiple cutaneous MCTs that develop concurrently or over time recently was characterized in dogs and is believed to occur in 9% to 22% of dogs with MCTs.[11,38,41,52] The available evidence suggests that dogs that have more than one concurrent MCT may have an outcome similar to that for dogs with only one tumor and that similar prognostic factors and advisable therapeutic approaches obtain for these patients.[38] Whether this phenomenon represents an atypical form of metastasis or the independent development of multiple, unrelated tumors is unclear, although one recent study demonstrated a clonal origin for two distant cutaneous tumors that arose over years.[115] The current World Health Organization (WHO) staging system for canine MCTs classifies dogs with multiple cutaneous tumors as having advanced stage (stage III) disease.[116] Given these recent findings, a revision of this staging scheme is in order.

Ancillary therapy for the systemic effects of MCTs related to degranulation sometimes is recommended. All or some of the effects of histamine release can be blocked by administering the H$_1$ blocker diphenhydramine (2 to 4 mg/kg given orally twice daily) and the H$_2$ blockers cimetidine (4 mg/kg given orally three

times daily) or ranitidine (2 mg/kg given twice daily given orally). Omeprazole (0.5 to 1 mg/kg given once daily given orally), a newer proton pump inhibitor, appears effective at controlling clinical signs of gastrointestinal ulceration associated with gastrinoma[117] and should be equally effective in MCTs. We generally reserve the use of these agents for cases in which (1) systemic signs or local signs (Darier's sign) of degranulation are present; (2) the tumor is likely to be entered or manipulated at surgery (i.e., cytoreductive surgery); and (3) treatment is undertaken where gross disease will remain and degranulation is likely to occur *in situ* (e.g., radiotherapy or chemotherapy for tumors that are not cytoreduced). These agents are not routinely used in cases in which wide surgical excision is to be performed without excessive manipulation of the tumor itself. For cases with active evidence of gastric or duodenal ulceration, the addition of sucralfate (0.5 to 1 g given orally three times daily) and occasionally misoprostol (2 to 4 μg/kg given orally three times daily) to histamine blockers is prudent. Some experimental data suggest that H_1 and H_2 blockers also could be beneficial for preventing or resolving histamine-mediated wound breakdown,[33] but this has not been systematically evaluated. Some have mentioned the use of protamine sulfate, a heparin antagonist, for intraoperative hemorrhage.[69]

As discussed previously, most canine MCTs express Kit, the receptor for stem cell factor, and 13% to 50% of canine MCTs harbor activating mutations in the juxtamembrane domain of the receptor, constitutively activating the receptor. Several studies have further shown that this mutation is associated with a higher histologic grade or more aggressive biologic behavior.[118] Recently, Liao and coworkers[119] demonstrated that small molecules that competitively inhibit the adenosine triphosphate (ATP) binding pocket of the catalytic domain of Kit kinase are capable of inhibiting Kit autophosphorylation and canine MCT proliferation and of inducing apoptosis *in vitro*.[119] A phase I clinical trial of one of these inhibitors, SU11654, recently was completed in tumor-bearing dogs. Although a secondary endpoint of the study, a response rate of 50% was observed in dogs with MCTs, and c-Kit mutation status was a very important predictor of response to therapy.[118] Further evaluation of this and other, similar compounds is underway.

FELINE MAST CELL TUMORS

Incidence and Risk Factors

MCTs are the second most common cutaneous tumor in the cat, accounting for approximately 20% of cutaneous tumors in this species in the United States.[2,4,6] The incidence of MCTs in cats appears to have increased dramatically since 1950.[2] MCTs appear to occur much less frequently in the United Kingdom (8% of all cutaneous tumors) than in the United States.[1]

Two distinct forms of cutaneous MCTs in the cat have been reported: (1) the more typical mastocytic MCTs, which are histologically similar to MCTs in dogs, and (2) the less common histiocytic MCTs, which have morphologic features characteristic of histiocytic mast cells. An overall mean age of 8 to 9 years is reported for cats with MCTs; however, the mastocytic and histiocytic forms occur at mean ages of 10 and 2.4 years, respectively.[2,4,120] According to several studies, Siamese cats appear to be predisposed to the development of MCTs of both histologic types.[2,4,120-122] The distinct histiocytic form of MCT in cats is reported to occur primarily in young Siamese cats (under 4 years of age), including two related litters.[121] In contrast to these reports, in another series of cases Siamese cats were not more likely to develop the histiocytic form than the mastocytic form.[4] The authors of the latter report found that only two of seven cases of histiocytic MCT in their hospital population were Siamese cats. Earlier studies reported a male predilection for development of MCTs,[120,123] but larger, more recent series have failed to confirm such a predilection.[2,4]

Visceral MCTs appear to be more common in the cat than in the dog, with up to 50% of cases of MCT occurring in visceral sites in some series.[2,124] A splenic form (sometimes referred to as *lymphoreticular MCT*) is the most common differential diagnosis for "splenic disease" in cats, accounting for 15% of submissions in a series of 455 pathologic specimens.[125] The mean age of affected cats is approximately 10 years, and no breed or gender predilection is known.[2] Intestinal MCT is the third most common primary intestinal tumor in cats, after lymphoma and adenocarcinoma.[2] No breed or gender predilection is known. Older cats appear to be at risk (mean age, 13 years), but cats as young as 3 years have been reported.[126] In contrast to dogs, cats with visceral MCT disease often do not have concurrent or historical evidence of a cutaneous MCT.

The etiology of feline MCT is unknown. Viruslike particles have been reported in tissue samples from feline MCTs, but these failed to grow in tissue culture and were not transmissible to other cats, mice, or hamsters.[127] No association with the feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) or feline infectious peritonitis (FIP) has been reported. A genetic predisposition has been proposed because of the high incidence of MCTs in the Siamese breed.

Pathology and Natural History

The granules present in feline MCTs stain blue with Giemsa stain and purple with toluidine blue.[1,2,4] They tend to appear more eosinophilic than their canine counterparts with hematoxylin and eosin stains. As in

the dog, granules in feline mast cells contain vasoactive substances such as heparin and histamine.[2,128] In culture, feline mast cells contain surface-bound immunoglobulins and are capable of secreting histamine, heparin, and probably other vasoactive compounds when appropriately stimulated.[128] Complications associated with degranulation of MCTs also can occur in the cat, including coagulation disorders, gastrointestinal ulceration, and anaphylactoid reactions.[2,123,129] In addition, feline mast cells have phagocytic capability and can endocytose erythrocytes both in experimental models and in clinical samples.[130]

As previously mentioned, feline cutaneous MCTs occur in two histologically distinct forms, referred to as *mastocytic* and *histiocytic* varieties (Table 19-7).[4,121,122] The mastocytic form can be further subdivided on the basis of histologic appearance into two categories, often referred to as *compact* and *diffuse*, which may be prognostically significant.[2,121,131] The compact form (reportedly accounting for 50% to 90% of cases in several series) is associated with a more benign behavior. The diffuse form is histologically more anaplastic and behaviorally more malignant. The histologic grading system described for canine MCTs provided no prognostic information for the cat in several series.[120,132] Immunohistochemical studies have been performed on feline MCTs; all the tumors were vimentin positive, and most were positive for alpha-1-antitrypsin.[133] Metastatic rates for cutaneous MCTs in cats vary considerably, with reported rates ranging from 0 to 22%.[2,120,122,134]

In studies in which behavior was separated by compact and diffuse histologies, the relative majority of cases that recurred or metastasized had the diffuse histotype.[131] From this information, it generally can be stated that most feline cutaneous MCTs are behaviorally benign.

The uncommon histiocytic form of feline MCT is more challenging to diagnose histologically.[121,122] Mast cells may comprise only 20% of the cells present, with the majority being sheets of histiocytes, which lack distinct cytoplasmic granules and are accompanied by randomly scattered lymphoid aggregates and eosinophils. One report, in contrast, readily demonstrated metachromatic granules in seven cases of the histiocytic subtype. These may initially be misdiagnosed as granulomatous nodular panniculitis or deep dermatitis. In cases in the literature with follow-up information, histiocytic MCTs regressed spontaneously over 4 to 24 months.[121,122]

With visceral (i.e., splenic and intestinal) forms of MCT in the cat, widespread dissemination and metastasis are much more common. Necropsy data on 30 cats with splenic MCTs revealed dissemination in the following organs: liver (90%), visceral lymph nodes (73%), bone marrow (40%), lung (20%), and intestine (17%).[2] Up to one third of patients have peritoneal and pleural effusions rich in eosinophils and mast cells. Peripheral blood mastocytosis is present in as many as 40% of cases.[2] In one clinical report of 43 cases, 23% had bone marrow involvement.[129] Two gross forms of splenic involvement are possible; a diffuse, smooth form and a less common nodular form (Figure 19-17).[135,136] In one report, 18% of cats with cutaneous MCTs went on to develop splenic disease.[2] Interestingly, in the face of widespread metastasis, long-term survival after splenectomy is common with splenic MCT (see section on treatment and prognosis). Intestinal MCT in cats is also associated with widespread dissemination and has a poor prognosis.[2,126] It more commonly involves the small intestine (equally divided among the duodenum, jejunum, and ileum); colonic involvement is reported in fewer than 15% of cases. Lesions can be solitary or multiple. Peritoneal effusion rich in mast cells can

TABLE 19-7	Histologic Classification of Mast Cell Tumors in Cats	
Type	**Subtype**	**Microscopic Description**
Mastocytic		
	Compact (well differentiated)	Homogeneous cords and nests of slightly atypical mast cells with basophilic round nuclei, ample eosinophilic cytoplasm, and distinct cell borders; eosinophils conspicuous in only half of cases.
	Diffuse (anaplastic)	Less discrete, infiltrated into subcutis; larger nuclei (> 50% cell diameter), 2 to 3 mitoses/high-power field; marked anisocytosis, including mononuclear and multinucleated giant cells; eosinophils more commonly observed.
Histiocytic		Sheets of histiocyte-like cells with equivocal cytoplasmic granularity, accompanied by randomly scattered lymphoid aggregates and eosinophils; some reports note granules lacking, others find granules readily demonstrable.

A   B
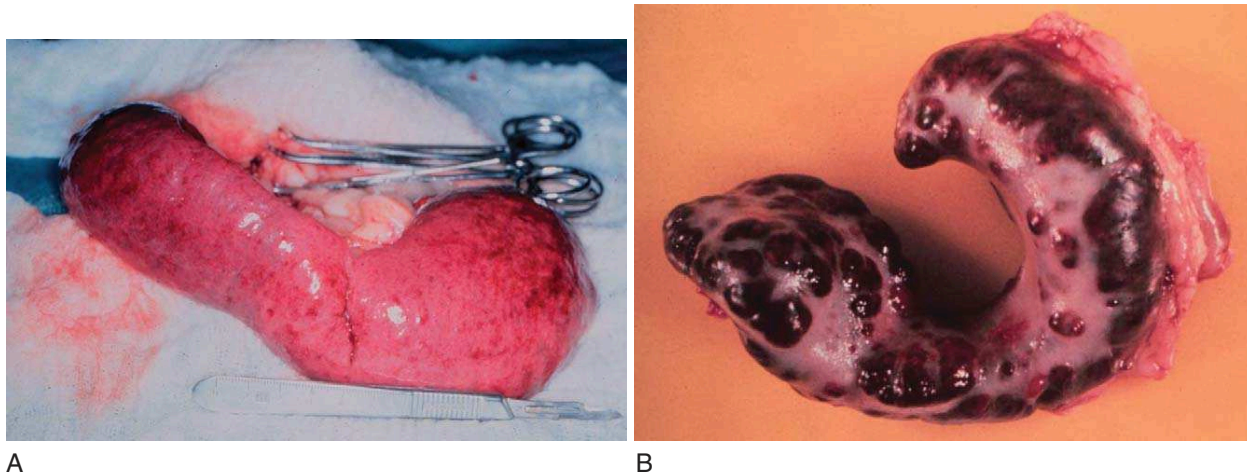
Figure 19-17

A, Diffuse, massive splenomegaly in a cat with splenic mast cell disease. **B,** The less common nodular form of splenic mast cell tumor in a cat.

occur. Unlike splenic MCT, peripheral mastocytosis is rarely associated with intestinal MCT, and only two reports of peripheral eosinophilia exist in the literature.[126] Metastasis to the mesenteric lymph nodes and the liver is common, followed less commonly by the spleen, lung, and bone marrow. Most animals die or are euthanized soon after diagnosis of MCT of the small intestine. Histologically, mast cells from intestinal lesions appear less differentiated than those of skin tumors, and cytoplasmic granules are less prominent. A cranial mediastinal form of MCT in cats also has been described.[2]

History and Clinical Signs

The typical feline cutaneous MCT is a solitary, raised, firm, well-circumscribed, hairless, dermal nodule 0.5 to 3 cm in diameter (Figure 19-18).[2,4,120,122] These lesions often are white, although a pink, erythematous form occasionally is seen. Approximately 20% are multiple, although one series from the United Kingdom reported multiple lesions in most cases.[1] Superficial ulceration is present in approximately one fourth of cases. Two other clinical forms have been described: a flat, pruritic, plaquelike lesion that resembles eosinophilic plaques, and discrete subcutaneous nodules.

Unlike in the dog, the head and neck are the most common sites for MCTs in the cat, followed by the trunk, limbs, and miscellaneous other sites (Figure 19-19).[2,4,120] Tumors on the head often involve the pinna near the base of the ear. They rarely occur in the oral cavity. Intermittent pruritus and erythema are common, and self-trauma or vascular compromise may

Figure 19-18

Typical solitary, raised, well-circumscribed, hairless, dermal cutaneous mast cell tumor in a cat. Excision was curative in this case.

result in ulceration. Darier's sign (erythema and wheal formation after mechanical manipulation of the tumor) has been reported in the cat.[123] Affected cats usually are otherwise healthy.

The spontaneously regressing histiocytic form of cutaneous MCT usually involves multiple, nonpruritic, firm, hairless, pink, and sometimes ulcerated subcutaneous nodules (Figure 19-20).[4,121] Affected animals are otherwise healthy.
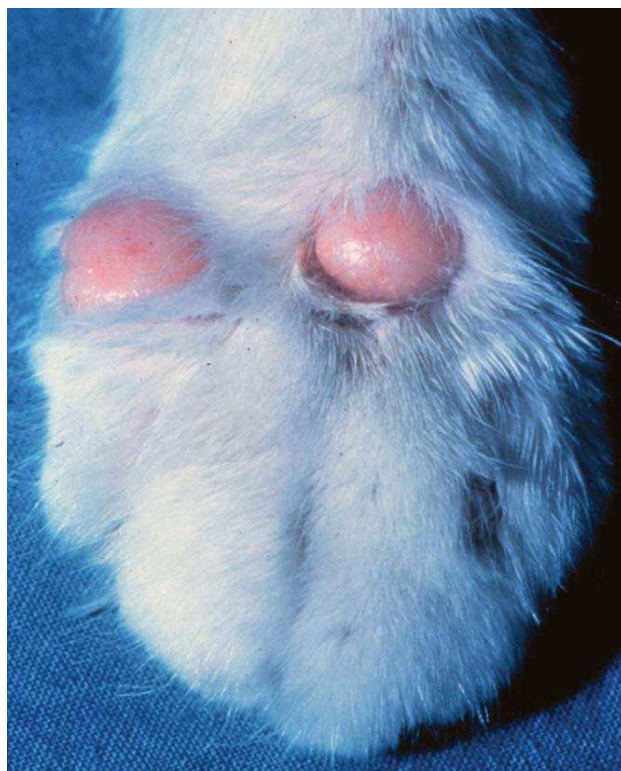
Figure 19-19

A, Multiple mast cell tumors on the head of a cat. Mast cell tumors most often develop on the head and neck in the cat. They often involve the area near the base of the ear. **B,** Mast cell tumor on the extremity in a cat. Mast cell tumors are less common on the limbs in cats. *(Photograph courtesy Dr. S. Helfand.)*
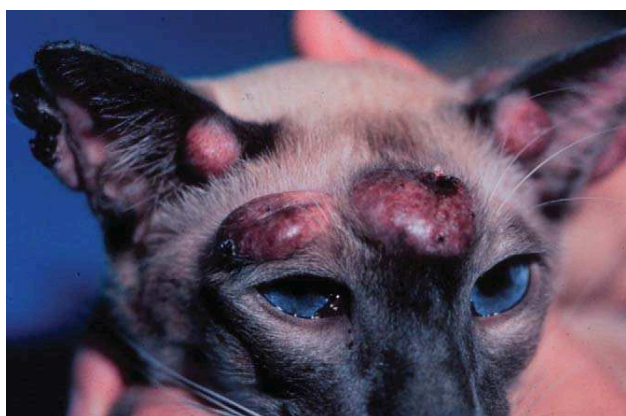
Figure 19-20

Histiocytic mast cell tumors on the head of a young Siamese cat. This form of mast cell tumor in cats typically regresses spontaneously, as was the case with this cat. *(Photograph courtesy Dr. K. Moriello.)*

Cats with disseminated forms of MCT may have signs of systemic illness. Depression, anorexia, weight loss, and intermittent vomiting are most commonly associated with splenic and intestinal MCT.[2,126,129,137] Abdominal palpation reveals massive splenomegaly in most cases, and occasionally the presence of peritoneal effusion is suggested in splenic MCT. Intestinal MCT often can be palpated as well. Diarrhea with or without bloody stools is commonly seen with the intestinal form, and fever may be present. Affected cats usually have been ill for several months. Signs related to the release of vasoactive components of mast cell granules, including gastrointestinal ulceration, uncontrollable hemorrhage, altered smooth muscle tone, hypotensive shock, and labored breathing, are more likely to be observed with systemic forms and are often episodic in nature. Labored breathing may also occur secondary to pleural effusion or anemia, which is present in approximately one third of cases of disseminated MCT in cats.[2,129]

Diagnostic Techniques and Workup

The diagnosis and staging of MCTs in cats are similar to those processes in the dog. FNA cytology usually is

diagnostic. This includes FNA of cutaneous lesions and splenic aspirates and thoracocentesis and abdominocentesis in the case of pleural and peritoneal effusions. Less frequently, intestinal mass aspirates are diagnostic. Tissue biopsy and histologic assessment typically are required for the histiocytic form of MCT.

When disseminated (e.g., splenic or intestinal) disease is suspected, CBC, buffy coat smear, bone marrow aspiration, coagulation profile, and serum biochemistry profile are helpful. One third of cats with visceral disease are anemic, and up to 50% of cats with splenic MCT have evidence of bone marrow and buffy coat involvement.[2,129] Peripheral mastocytosis can be striking; peripheral mast cell counts up to 32,000 cells/μl have been reported.[2] Unlike the splenic form, intestinal MCT is not commonly associated with peripheral mastocytosis, but eosinophilia has been reported.[126] In one report of 43 cats with splenic MCT, 90% had laboratory evidence of coagulation abnormalities. Although this rarely was clinically significant, knowledge of its presence should allow preoperative precautions to be taken if surgery is contemplated.[129] Hyperglobulinemia also has been reported in cats with splenic MCT, the cause of which remains unknown. Other common differential diagnoses for splenomegaly in the cat include lymphoma, myeloproliferative disease, accessory spleen, hemangiosarcoma, hyperplastic nodules, and splenitis.[125] The two most common differential diagnoses for intestinal masses in aged cats are lymphoma and adenocarcinoma.[2]

Thoracic and abdominal radiographs can confirm pleural and peritoneal effusions, which are present in up to one third of cats with splenic MCT.[2] Abdominal US may be required to determine the extent of intestinal involvement and lymph node size and to detect visceral dissemination. Although most intestinal MCTs are either palpable or visible on abdominal radiographs or ultrasound scans, exploratory celiotomy ultimately may be required for definitive diagnosis.

Treatment and Prognosis

Surgery is the treatment of choice for the mastocytic form of cutaneous MCT in the cat. As previously discussed, most of these lesions are behaviorally benign, and wide surgical margins may not be as critical as in the dog. This is fortunate, because most of these tumors occur on the head, where such margins would be difficult to achieve. The incidences of local recurrence and systemic spread vary widely in the literature. Local recurrence rates of 0 to 24% after surgical excision have been reported.[2,120,122,132,134] The incidence of systemic spread after surgical excision varies from 0 to 22%.[2,120,122,134] Recurrence, when it occurs, usually is noted within 6 months. For histologically anaplastic (i.e., diffuse) mastocytic tumors, a more aggressive approach similar to that used for canine MCTs may be prudent, because higher rates of recurrence and metastasis are associated with this type. After biopsy confirmation, conservative resection or a "wait and see" approach may be taken with the histiocytic form in young cats with multiple masses, because most are reported to regress spontaneously.[2,4,121]

Relatively little is known about the effectiveness of nonsurgical therapy for cutaneous MCTs in the cat. Responses to lomustine (CCNU) have been reported,[138] and the authors also have observed evidence of clinical activity in cats treated with prednisone and VBL. The response to steroids in cats with the histiocytic form is equivocal.[121]

Cats with splenic MCT usually benefit from splenectomy. Surprisingly, even in the face of significant bone marrow and peripheral blood involvement, long-term survival with good quality of life is the norm after splenectomy, with median survival times of 12 to 19 months reported.[2,129,137,139,140] Anorexia, significant weight loss, and male gender have been associated with a worse prognosis. Frequent re-evaluation and buffy coat smears are used to follow response in such cases. Rarely does the peripheral mastocytosis resolve, but it often declines significantly, and a subsequent rise can serve as a marker of progression. Adjunctive treatment with combination chemotherapy protocols, including prednisone, vincristine, cyclophosphamide, and methotrexate, have been attempted in a limited number of cases but do not appear to increase survival times.[2] The role of adjuvant CCNU or VBL in cats after splenectomy has not been established and is currently being evaluated. The use of ancillary drugs to combat the effects of vasoactive amines (see discussion under canine MCT) may transiently abrogate clinical signs.

The intestinal form of feline MCT has a poor prognosis. Metastasis at the time of diagnosis is common, and most cats die or are euthanized soon after. If surgery is possible, wide surgical margins (i.e., including 5 to 10 cm of normal bowel on either side of the lesion) are recommended, because the tumor often extends histologically well beyond visible gross disease.[2] Adjuvant therapy of some form is reasonable in these cases.

COMPARATIVE ASPECTS OF MAST CELL TUMORS

Mast cell cancer is rare in humans.[141] As in dogs and cats, a wide range of syndromes exist, from hyperplastic syndromes to malignant visceral forms and mast cell leukemia. The most common human mast cell neoplasm is a multiple cutaneous form that occurs in infancy, known as *urticaria pigmentosa*.[142] It is similar to mast cell disease described in kittens[2,121,122] and a puppy[7] in that it arises during infancy and regresses spontaneously at the

onset of puberty. It is not associated with a histiocytic histology, as in the kitten. Other forms of MCT in humans include benign systemic mastocytosis, malignant mastocytosis, and mast cell leukemia. Approximately one third of patients with urticaria pigmentosa go on to develop malignant mastocytosis, a uniformly fatal disease. As in veterinary patients, many human patients are susceptible to systemic symptoms caused by vasoactive substances released during degranulation of neoplastic mast cells. They often benefit symptomatically from H_1 and H_2 blocker therapy. Because of the rarity of this condition, no standard treatment protocols are available for malignant mast cell disease in humans. Corticosteroids, daunorubicin, and chlorambucil are reported to be effective in some cases of human mastocytosis,[143] but meaningful case series and clinical trials are lacking. Novel treatments that show promise in humans include interferon-alpha,[144] the nucleoside analog 2-chloroadenosine (cladribine),[145,146] and Kit tyrosine kinase inhibitors.[147]

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20

Soft Tissue Sarcomas

Julius M. Liptak and Lisa J. Forrest

INCIDENCE AND RISK FACTORS

Soft tissue sarcomas are a heterogeneous population of mesenchymal tumors that constitute 15% and 7% of all skin and subcutaneous tumors in the dog and cat, respectively.[1] The annual incidence of soft tissue sarcomas in companion animals is about 35 per 100,000 dogs at risk and 17 per 100,000 cats at risk.[2] In dogs, sarcomas have been associated with radiation, trauma, foreign bodies, orthopedic implants, and the parasite *Spirocerca lupi*.[3-9]

Most soft tissue sarcomas are solitary tumors in middle to older aged dogs and cats. There is no specific breed or sex predilection for soft tissue sarcomas with the possible exception of synovial cell sarcomas in dogs. Earlier reports indicate a slight male predilection;[10,11] however, in one study males and females were equally represented.[12] Another exception is the occurrence of rhabdomyosarcoma tumors in young dogs.[13] Soft tissue sarcomas tend to be overrepresented in large-breed dogs.

PATHOLOGY AND NATURAL HISTORY*

Soft tissue sarcomas are a heterogeneous group of tumors whose classification is based on similar pathologic appearance and clinical behavior. Sarcomas arise from mesenchymal tissues and have features similar to the cell type of origin (Table 20-1).* These tumors originate in connective tissues, including muscle, adipose, neurovascular, fascial, and fibrous tissue, and they can give rise to benign and malignant entities. Only malignant soft tissue sarcomas will be covered in this chapter. Malignant neoplasms in this category include fibrosarcoma, peripheral nerve sheath tumor (PNST, also known as malignant schwannoma, neurofibrosarcoma, or hemangiopericytoma), myxosarcoma, undifferentiated sarcoma, liposarcoma, histiocytic

*Tables 20-1 and 20-2 and general pathology description courtesy of Dr. Barbara Powers, Colorado State University.

sarcoma (or malignant fibrous histiocytoma), and rhabdomyosarcoma (Box 20-1).[14,15] The term *soft tissue sarcoma* generally excludes those tumors of hematopoietic or lymphoid origin. Hemangiosarcoma (covered briefly here), mast cell sarcoma, oral sarcoma, osteosarcoma, and chondrosarcoma are covered separately in other chapters. Feline sarcomas and vaccine-associated sarcomas are covered in a separate section at the end at this chapter.

Soft tissue sarcomas have several important common features with regard to their biologic behavior. They may arise from any anatomical site in the body, although skin and subcutaneous sites are most common.

- They tend to appear as pseudoencapsulated soft to firm tumors but have poorly defined histologic margins or infiltrate through and along facial planes. They are locally invasive.
- Local recurrence after conservative surgical excision is common.
- Sarcomas tend to metastasize hematogenously in up to 20% of cases.
- Regional lymph node metastasis is unusual (except for synovial cell sarcoma).[10]
- Histopathologic grade is predictive of metastasis, and resected tumor margins predict local recurrence.[16]
- Measurable or bulky (>5 cm in diameter) tumors generally have a poor response to chemotherapy and radiation therapy.

Soft tissue sarcomas present a diagnostic challenge. Many of these tumors have histologic patterns with overlapping features not only among themselves but also with a variety of other neoplasms with different histogenesis. The development of immunocytochemical procedures, the availability of monoclonal antibodies and polyclonal antibodies to various tissue markers, and tissue microarray technology have improved diagnosis of soft tissue sarcomas in human pathology and to a limited degree in veterinary pathology.[12,17-22]

The histologic nomenclature for some sarcomas may vary from pathologist to pathologist. Before the initiation of the appropriate therapy for the treatment of soft tissue sarcomas, it is necessary to know the species,

TABLE 20-1   Histiogenic Classification and Metastatic Potential of Canine Soft Tissue Sarcomas

Tissue of Origin	Benign	Malignant	Primary Sites	Metastatic Potential	Metastatic Sites
Fibrous tissue	Fibroma	Fibrosarcoma	Extremity and oral cavity	Low-to-moderate[a]	Lungs
Nervous tissue	—	Peripheral nerve sheath tumor	Extremity	Low-to-moderate[a]	Lungs
Myxomatous tissue	Myxoma	Myxosarcoma	Extremity and joints	Low-to-moderate	Lungs
Adipose tissue	Lipoma	Liposarcoma	Extremity and ventrum ± abdominal or thoracic cavity	Low-to-moderate	Lungs ± liver, spleen, bone
Skeletal muscle	Rhabdomyoma	Rhabdomyosarcoma	Tongue, larynx, heart, and urinary bladder	Low-to-moderate	Lungs ± liver, spleen, kidneys
Smooth muscle	Leiomyoma	Leiomyosarcoma[b]	Gastrointestinal ± spleen, liver, vulva and vagina, and subcutaneous tissue	Moderate	Lymph nodes, liver ± spleen, kidneys, peritoneum
Synovial tissue	Synovioma	Synovial cell sarcoma[b]	Joints	Moderate-to-high[a]	Lymph nodes and lungs
Histiocytic cells	Histiocytoma	Histiocytic sarcoma[b]	Extremity	Moderate-to-high	Lymph nodes ± lungs, spleen, liver, kidneys
Lymph vessels	Lymphangioma	Lymphangiosarcoma[b]	Extremity	Moderate	Lymph nodes
Blood vessels	Hemangioma	Hemangiosarcoma[b]	Spleen, heart, liver ± skin, muscle, bone, kidneys, and retroperitoneum	High	Lungs, liver, lymph nodes, distant dermal sites

[a]Dependent on histologic grade.
[b]Atypical soft tissue sarcoma with higher metastatic rate and higher rate of metastasis to regional lymph nodes.

Box 20-1	Types of Soft Tissue Sarcomas

Soft tissue sarcoma	**Other soft tissue sarcoma**	**Non–soft tissue sarcoma**
Fibrosarcoma	Leiomyosarcoma	Histiocytic sarcoma
Peripheral nerve sheath tumor[a]	Rhabdomyosarcoma	Hemangiosarcoma
Liposarcoma	Synovial cell sarcoma	Osteosarcoma
Myxosarcoma	Lymphangiosarcoma	Chondrosarcoma
Malignant mesenchymoma		Melanoma
		Lymphosarcoma (lymphoma)

[a]*Peripheral nerve sheath tumors include tumors previously classified as hemangiopericytoma, malignant schwannoma, and neurofibrosarcoma. Tumors listed under "Soft Tissue Sarcoma" have similar biologic behavior characterized by local aggression and low-to-moderate metastatic potential. Tumors listed under "Other Soft Tissue Sarcomas" are atypical soft tissue sarcomas because of different location (e.g., leiomyosarcoma and synovial cell sarcoma) or higher metastatic rate (leiomyosarcoma, rhabdomyosarcoma, lymphangiosarcoma ± synovial cell sarcoma). Some sarcomas not considered as soft tissue sarcomas are discussed in this chapter because of similarities in location to other soft tissue sarcomas (e.g., histiocytic sarcoma and dermal hemangiosarcoma).[125]*

histologic type of sarcoma, size, site, histologic grade, and the stage of disease. The majority of soft tissue sarcomas can occur in both low-grade and high-grade histologic forms. Histologic grading (e.g., low, intermediate, high, or I, II, III) is assigned after histologic characterization from large biopsy specimens (Table 20-2).

SPECIFIC TUMOR TYPES

Soft tissue sarcoma is a collective term used to describe a number of different types of tumors with similar histologic features and biologic behavior. The histiogenesis of soft tissue sarcomas is controversial and can be difficult to differentiate on the basis of routine histologic and immunohistochemical analysis. Some pathologists have recommended the use of a more generic term, such as soft tissue sarcoma or *spindle cell tumor of canine soft tissue*, because of the difficulty in differentiating tumors such as fibrosarcoma, PNST, and hemangiopericytoma.[23] Moreover, histologic distinction of tumor type is not clinically important because most soft tissue sarcomas have a similar biologic behavior, (i.e., locally aggressive with a low-to-moderate distant metastatic rate). Some types of soft tissue sarcomas will be covered briefly in this chapter, such as hemangiosarcoma, lymphangiosarcoma, and synovial cell sarcoma, which are atypical because their biologic behavior is different with a higher rate and different distribution of metastasis.

Tumors of Fibrous Origin

Nodular fasciitis (fibromatosis, pseudosarcomatous fibromatosis)

Nodular fasciitis is a benign non-neoplastic lesion arising from the subcutaneous fascia or superficial portions

TABLE 20-2	Soft Tissue Sarcoma Grading System[16]		
Score	**Differentiation**	**Mitosis**[a]	**Necrosis**
1	Resembles normal adult mesenchymal tissue	0–9	None
2	Specific histologic subtype	10–19	<50% necrosis
3	Undifferentiated	>20	>50% necrosis

Grade I = cumulative score of ≤ 4 for the three categories, Grade II = cumulative score of 5 to 6, and Grade III = cumulative score of ≥ 7.

[a]*Mitosis is calculated as the number of mitotic figures per 10 high-power fields.*

of the deep fascia in dogs. These lesions are usually nodular, poorly circumscribed, and very invasive.[24] Histologically, nodular fasciitis is characterized by large plump or spindle-shaped fibroblasts in a stromal network of variable amounts of collagen and reticular fibers with scattered lymphocytes, plasma cells, and macrophages.[24] The morphologic and pathologic characteristics of nodular fasciitis can result in these lesions being misdiagnosed as fibrosarcoma. Infantile desmoid-type fibromatosis is a variant of nodular fasciitis and is characterized by fibroblast proliferation with a dense reticular fiber network and mucoid material.[25] Wide excision of both nodular fasciitis and infantile desmoid-type fibromatosis lesions is usually curative.[26] Local recurrence is possible with incomplete resection. These tumors do not metastasize.[24]

Fibrosarcoma

Most fibrosarcomas arise from the skin, subcutaneous tissue, or oral cavity and represent malignant fibroblasts. Tumors can be well differentiated, exhibiting spindle shaped tumor cells with scant cytoplasm. The more anaplastic tumor is very cellular with closely packed spindle-shaped fibroblasts showing many mitotic figures and marked cellular pleomorphism.[27] Tumors tend to occur in older dogs and cats with no breed or sex predilection; however, one reference states a higher predilection in golden retrievers and Doberman pinschers.[28] A unique form, *histologically low-grade, yet biologically high-grade tumors*, is seen in the oral cavity, has a tendency to grow to quite a large size, and invades deeper structures, including bone. Metastasis can be seen in up to 20% of the cases.[19] Metastasis is rare, but these tumors are infiltrative with microscopic tumor cells invading along fascial planes and often recur after surgical excision.

Tumors of the Peripheral Nerves

Peripheral nerve sheath tumor

The PNSTs are malignant tumors of nerve sheath origin and have been referred to as neurofibrosarcoma, malignant schwannoma, and hemangiopericytoma. The confusion regarding hemangiopericytoma is the fact that blood vessel pericyte origin has yet to be proven in dogs. Most hemangiopericytomas will have features of nerve sheath tumors histologically.[27] Malignant PNSTs will stain positive with S100 and vimentin, indicating peripheral nerve origin.[29]

Regardless of the nomenclature, these tumors can occur anywhere in the body. A PNST may involve nerves away from the brain or spinal cord (peripheral group), or they may involve nerves immediately adjacent to brain or spinal cord (root group) or the brachial or lumbosacral (plexus group).[30] The true peripheral form is much more treatable than the plexus or root form. Despite appearing encapsulated at surgery, these tumors are similar to fibrosarcoma and occur as poorly defined tumors without histologic encapsulation. Most of the tumors are adherent to deeper tissues and infiltrate underlying fascia, muscle, and skin. Although these tumors are considered malignant, they have a modest metastatic rate. As with fibrosarcoma, local recurrence for PNST is common following conservative surgery. They tend to grow slowly and can range in size from 0.5 cm to more than 10 to 12 cm in diameter. In some cases, they can easily be confused with lipomas on initial clinical examination.[27]

Peripheral nerve sheath tumors located in the axial region may result in the compression of nerves. The vast majority of cases will show signs of unilateral lameness, muscle atrophy, paralysis, and pain.[30] They can invade the spinal cord, and about 50% will invade the cord if a grade III tumor is diagnosed.[30] However, local disease usually limits survival before metastasis occurs.

Fibrohistiocytic Tumors

Benign histiocytoma

Benign histiocytomas are common cutaneous neoplasms of dogs younger than 3 years old.[31] Histiocytomas are usually solitary but a multiple form appears to be more common in shar-pei dogs.[31] Morphologically, multiple histiocytomas can be difficult to differentiate from epidermotropic (or mycosis fungoides) and nonepidermotropic cutaneous lymphoma, and immunohistochemistry is required for definitive diagnosis.[31] Solitary benign histiocytomas can undergo spontaneous regression within 6 weeks, but regression can be delayed for up to 10 months, particularly in dogs with multiple benign histiocytomas.[31]

Localized histiocytic sarcoma

Histiocytic sarcomas are malignant tumors originating from histiocytic cells. The diagnosis of histiocytic sarcoma can be confusing and is controversial. Macrophages and dendritic cells are histiocytic cells that share a common bone marrow precursor but differentiate into two phenotypically separate cell lineages with different tissue distribution and function.[32] Macrophages are phagocytic and essential components of the innate immune system. Dendritic cells are poorly phagocytic and specialize in processing and presenting antigens to T lymphocytes to evoking an antigen-specific immune response.[32] The immunohistochemical analysis of histiocytic sarcomas suggests these tumors originate from the dendritic antigen-presenting cell lineage.[31,33]

There are two distinct types of histiocytic sarcoma based on clinical presentation and biologic behavior: localized and disseminated. Localized histiocytic sarcomas are locally aggressive and have a moderate-to-high metastatic potential.[32] Immunohistochemistry is required for definitive diagnosis, with recommended stains including CD1b, CD1c, CD11c, CD18, and MHC II.[32-34] Unlike typical soft tissue sarcomas, localized histiocytic sarcomas have a higher metastatic potential and tend to metastasize to regional lymph nodes, while other metastatic sites include the lungs, spleen, liver, and kidneys.[32,33] Malignant fibrous histiocytomas (MFHs), a fibrohistiocytic sarcoma with a multinucleated giant cell component and the most commonly diagnosed soft tissue sarcoma of the extremity in humans,[35] may be analogous to localized histiocytic sarcomas, but *MFH* is rarely used and the latter term is preferred by most veterinary pathologists.[33,36]

Flat-coated retriever, rottweiler, golden retriever, and Labrador retriever are predisposed dog breeds, although

cats and other dog breeds have been reported.[32,33] Middle-aged to older dogs are usually affected, and there is no sex predilection. Localized histiocytic sarcomas usually present as a solitary cutaneous or subcutaneous mass on the extremity, although other locations have been reported, such as the spleen, lung, and periarticular tissue.[32,33,37-40] The hemophagocytic form of histiocytic sarcoma, in which there is histologic evidence of red blood cell, leukocyte, and tumor cell phagocytosis, is more common in splenic histiocytic sarcomas and represents a more aggressive form of disease.[31] Thorough clinical staging, consisting of thoracic radiographs, abdominal ultrasound, regional lymph node aspiration or biopsy, and bone marrow cytology, should be performed to differentiate localized from disseminated histiocytic sarcoma.[39,40]

Surgery is recommended for treatment of the local disease; however, an aggressive surgical approach is required as tumor invasion of the underlying deep dermis, skeletal muscle, and fascia is common.[33] Radiation therapy may be indicated for incompletely resected localized histiocytic sarcomas as round cells are radiation-sensitive and a complete response has been reported in one dog with a histiocytic sarcoma of the tongue.[33] Furthermore, local tumor control is better following irradiation of incompletely resected MFH in humans.[41] The role of adjunctive chemotherapy is unknown but, because of the metastatic potential and cell of origin, doxorubicin- or lomustine-based protocols may be warranted.

The prognosis for dogs with localized histiocytic sarcoma of the skin and subcutis is unknown but, in one small retrospective series, local tumor recurrence or metastasis was not observed in all five dogs with long-term follow-up treated with wide surgical resection and no adjunctive therapy.[33] In contrast, the prognosis for dogs with localized histiocytic sarcoma of the internal organs, such as the spleen, is poor with a median survival time of 1 month and a 0% to 20% 1-year survival time.[33,37,42] Poor prognostic factors in dogs with splenic histiocytic sarcomas include lymphoid-to-fibrohistiocytic ratio, mitotic index, and histologic grade.[37,42] Dogs with a lymphoid-to-fibrohistiocytic ratio of ≥40% have a 1-year survival probability of 87% compared to 55% if the ratio is < 40%.[42] Dogs with grade III fibrohistiocytic nodules have a 1-year survival rate of 32%, which is significantly worse than the 1-year survival rate of 57% and 61% for dogs with grade I and II lesions, respectively.[42]

Disseminated histiocytic sarcoma (malignant histiocytosis)

Disseminated histiocytic sarcomas are multicentric tumors, usually involving the lymph nodes, bone marrow, lungs, spleen, and liver.[32,33] Disseminated histiocytic sarcoma is also known as malignant histiocytosis.[33]

Predisposed dog breeds include Bernese mountain dogs, rottweilers, golden retrievers, Labrador retrievers, and flat-coated retrievers.[32,34] There is no age or sex predilection.[32,33] Unlike localized histiocytic sarcomas, dogs with disseminated histiocytic sarcomas are often symptomatic with clinical signs dependent on the affected organ systems.[32] Anemia, thrombocytopenia, and leukocytosis are relatively common and autoimmune hemolytic anemia is observed in up to 20% of affected dogs.[43] Localized and disseminated histiocytic sarcomas are differentiated by clinical presentation and staging as they cannot be differentiated with ultrasonographic appearance, histology, or immunohistochemistry.[32,39,40] Treatment is usually unrewarding, although durable partial responses have been observed with chemotherapy using lomustine.[44] The prognosis is poor with a median survival time of 128 days and a 0% to 30% 1-year survival rate.[43,44] Poor prognostic factors include thrombocytopenia, hypoproteinemia, and histologic evidence of giant cells.[43,44]

Tumors of Adipose Tissue

Lipoma

Lipomas are benign tumors of adipose tissue. Variants of lipomas have been reported and include angiolipoma and angiofibrolipoma.[45] Lipomas are relatively common in older dogs, especially in subcutaneous locations, and are rarely symptomatic. Lipomas can also occur in the thoracic cavity, abdominal cavity, spinal canal, and the vulva and vagina of dogs, and they can cause clinical abnormalities secondary to either compression or strangulation.[46-54] Parosteal and infiltrative lipomas have also been reported, and these tumors can have a more aggressive behavior despite their benign histologic appearance.[55-60] Marginal resection is recommended for lipomas that interfere with normal function; however, the majority are asymptomatic and do not require surgical intervention. Lipomas can be differentiated from liposarcomas based on morphologic and histologic appearance. Histologically, lipomas have indistinct nuclei and cytoplasm resembling normal fat, whereas liposarcomas are characterized by increased cellularity, distinct nuclei, and abundant cytoplasm with one or more droplets of fat.[52] Surgical resection is usually curative, but local recurrence has been reported.[53]

Intermuscular Lipoma

Intermuscular lipomas are a variant of the subcutaneous lipoma and are located in the intermuscular region of the caudal thigh of dogs, particularly between the semitendinosus and semimembranosus muscles (Figure 20-1).[61] Clinically, intermuscular lipomas appear as a slow-growing, firm, and fixed mass in the

Figure 20-1

An intermuscular lipoma arising from between the semitendinosus and semimembranosus muscles. Surgical dissection and removal is curative.

Figure 20-2

A CT image of a dog with an infiltrative lipoma of the left chest wall (*arrows*). Despite being a benign tumor, aggressive surgical resection and possibly postoperative radiation therapy are required to control the local tumor.

caudal thigh region, and may occasionally cause lameness.[61] Cytologic analysis of fine-needle aspirates is usually diagnostic. The recommended treatment is surgical resection, involving blunt dissection and digital extrusion, and placement of either a Penrose or negative-suction drain. Seromas are a common complication in dogs in which a drain is not used. The prognosis is excellent with no recurrence reported in 11 dogs following surgical removal.[61]

Infiltrative lipoma

This uncommon tumor is composed of well-differentiated adipose cells without evidence of anaplasia. These tumors cannot be readily distinguished from the more common simple lipoma by cytology or small biopsy specimens. They are considered benign and do not metastasize. However, infiltrative lipomas are locally aggressive and commonly invade adjacent muscle, fascia, nerve, myocardium, joint capsule, and even bone (Figure 20-2).[56,62,63] Computed tomography is used to better delineate these tumors; however, they do not contrast enhance and differentiating infiltrative lipomas

from normal fat is a problem.[61] One retrospective analysis of 16 cases reported a 4:1 female-to-male ratio.[58] Aggressive treatment, including amputation, may be necessary for local control. Radiation therapy can be considered either alone or in combination with surgical excision.[60]

Liposarcoma

Liposarcomas are uncommon malignant tumors originating from lipoblasts in older dogs.[64] Liposarcomas do not arise from malignant transformation of lipomas. Specific causes are not known, but foreign body-induced liposarcoma has been reported in one dog.[5] There is no breed or sex predilection.[64] They are commonly reported in subcutaneous locations, especially along the ventrum and extremities, but can also occur in other primary sites such as bone and the abdominal cavity. Liposarcomas are differentiated from lipomas based on morphologic appearance and cytologic characteristics with liposarcomas usually firm and poorly circumscribed. They are locally invasive with a low metastatic potential. Metastatic sites include the lungs, liver, spleen, and bone.[27,64]

The prognosis for liposarcoma is good with appropriate surgical management. The median survival time following wide surgical excision is 1188 days, and this is significantly better than either marginal excision or incisional biopsy, which have median survival times of

649 days and 183 days, respectively (Figure 20-3).[64] Liposarcoma is histologically classified as well differentiated, myxoid, round cell (or poorly differentiated), pleomorphic, or dedifferentiated. This classification scheme has clinical and prognostic importance in humans as pleomorphic liposarcomas have a high metastatic rate, myxoid liposarcomas are more likely to metastasize to extrapulmonary soft tissue structures, and well-differentiated liposarcomas are unlikely to metastasize.[35,65,66] In a large retrospective study in dogs, histologic subtype was not prognostic, but metastatic disease was more common in dogs with pleomorphic liposarcomas.[64]

Tumors of Smooth Muscle

Leiomyoma and leiomyosarcoma

Leiomyomas and leiomyosarcomas are tumor arising from smooth muscle cells. The gastrointestinal tract is most commonly affected, but other primary sites include the spleen, liver, genitourinary tract, retroperitoneal space, vessel wall, and subcutaneous tissue.[67-70] Paraneoplastic syndromes associated with smooth muscle tumors, particularly gastrointestinal leiomyomas and leiomyosarcomas, include hypoglycemia, nephrogenic diabetes insipidus, and secondary erythrocytosis.[68,71-74]

Leiomyomas are benign and usually small, localized, and well encapsulated. Leiomyomas of the vagina and vulva are often pedunculated, protrude from the vulva, and are hormonally dependent. Ovariohysterectomy is recommended in the management of dogs with vulval or vaginal leiomyomas.

Leiomyosarcomas are malignant tumors with a moderate metastatic potential depending on the primary site.[68,75] The metastatic rate for dogs with hepatic leiomyosarcoma is 100% but is usually less than 50% for other primary intra-abdominal sites and 0% for dermal smooth muscle tumors.[68,74,76,77] Regional lymph nodes, mesentery, and liver are the most common metastatic sites for gastrointestinal leiomyosarcoma, although others include the spleen, kidneys, and peritoneum.[68,74-77]

Leiomyosarcoma is the second most common gastrointestinal tumor in dogs and has a predilection for the jejunum and cecum, but any region of the gastrointestinal tract can be affected from the esophagus to the rectum.[68,74-76,78] The term *gastrointestinal stromal tumors* is preferred by some investigators as immunohistochemical analysis has shown that these tumors can originate from interstitial cells of Cajal (true gastrointestinal stromal tumor) or vascular or gastrointestinal smooth muscle cells (leiomyosarcoma).[75,78] Leiomyosarcomas have strong immunoreactivity to actin and desmin and rarely stain positively with c-kit, CD34, or S-100 protein. In contrast, true gastrointestinal
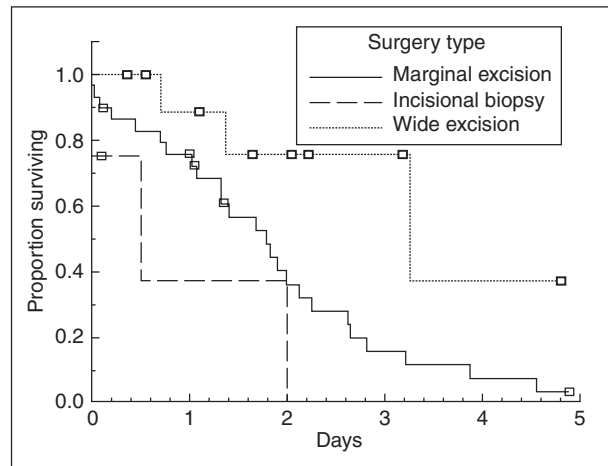
Figure 20-3

Kaplan-Meier survival curve of 56 dogs with liposarcoma treated with either incisional biopsy, marginal resection, or wide excision. The median survival time is significantly longer, at 1188 days, following wide surgical resection than less aggressive techniques. *(Reprinted with permission from J. L. Baez, M. J. Hendrick, F. S. Shofer, et al., "Liposarcomas in dogs: 56 cases (1989–2000)," J Am Vet Med Assoc 224:887, 2004.)*

stromal tumors are consistently associated with mutations of the tyrosine kinase receptor c-kit and will have strong immunoreactivity to c-kit and CD34 with variable reactivity to actin, desmin, and S-100 protein.[75,78]

Older dogs are more commonly affected, and there is no sex or breed predisposition.[68,74-76] In contrast, there is a male predisposition for gastrointestinal leiomyomas, and these tumors have a predilection for the stomach rather than the jejunum and cecum.[75,79] Presenting signs can include inappetance, weight loss, vomiting, diarrhea, polyuria, polydipsia, anemia, and hypoglycemia.[68,74-76]

Surgical resection is the recommended treatment for dogs with leiomyosarcoma. Intestinal perforation with localized to diffuse peritonitis is relatively common in dogs with gastrointestinal leiomyosarcoma and is reported in up to 50% of cases.[74] Local tumor control is good following complete resection, but recurrence has been reported following incomplete resection of a gastric leiomyoma and cutaneous leiomyosarcomas.[77,79]

Prolonged survival and possibly cure has been reported following surgery in dogs with gastrointestinal leiomyosarcoma.[67,68,72] Prognostic factors in humans with gastrointestinal stromal tumors include tumor size, metastasis, and histologic criteria such as tumor necrosis, number of mitotic figures, and proliferating cell nuclear antigen (PCNA) index;[80] however, prognostic factors have not been investigated in dogs with leiomyosarcoma. The median survival time for dogs with gastrointestinal leiomyosarcoma surviving the

immediate postoperative period is up to 21.8 months with 1-, 2-, and 3-year survival rates of 75%, 66%, and 60%, respectively.[68,74,76] In addition, metastasis did not have a negative impact on survival time in one report with a 21.7-month mean survival time for dogs with documented metastasis at the time of surgery.[74] However, other investigators have found that metastasis significantly decreases survival time.[76] The median survival time is 8 months for dogs with splenic leiomyosarcoma and 0 months for dogs with hepatic leiomyosarcoma.[68]

Tumors of Skeletal Muscle

Rhabdomyosarcoma

Rhabdomyosarcomas are rare malignant tumors originating from myoblasts or primitive mesenchymal cells capable of differentiating into striated muscle cells.[81] In dogs, rhabdomyosarcomas are most frequently reported to arise from skeletal muscle of the tongue, larynx, myocardium, and urinary bladder. They are locally invasive with a low-to-moderate metastatic potential. Metastatic sites include the lungs, liver, spleen, kidneys, and adrenal glands.[81]

Rhabdomyosarcomas are histologically classified as embryonal, botryoid, alveolar, and pleomorphic. The histologic diagnosis of rhabdomyosarcoma is difficult and immunohistochemical staining for vimentin, skeletal muscle actin, myoglobin, myogenin, and MyoD may be required for definitive diagnosis.[82] Embryonal rhabdomyosarcomas have a predilection for the head and neck region, such as the tongue, oral cavity and larynx, of older dogs. In contrast, botryoid rhabdomyosarcoma commonly arises in the urinary bladder of young, female large-breed dogs, with Saint Bernard dogs overrepresented.[81] Botryoid tumors are characterized by their grapelike appearance. The histologic classification scheme for rhabdomyosarcoma has prognostic significance in humans, but this has not been investigated in dogs. In humans, botryoid rhabdomyosarcoma has a good prognosis, embryonal rhabdomyosarcoma an intermediate prognosis, and alveolar rhabdomyosarcoma has a poor prognosis.[81,83]

Rhabdomyosarcomas, particularly those involving the extremities, have a high rate of metastasis in humans with major metastatic sites including the lungs, lymph nodes, and bone marrow. The metastatic potential and prognosis in dogs with rhabdomyosarcoma have not been determined because the condition is rarely diagnosed and even more rarely treated with curative intent. However, disease-free and overall survival times have been encouraging in the few dogs treated with surgical resection ± radiation therapy and chemotherapy.[84-88] In humans, in contrast to many other types of soft tissue sarcomas, multimodality treatment with surgery, radiation therapy, and chemotherapy has significantly improved survival rates,

particularly in children with embryonal and botryoid rhabdomyosarcoma.[35,66,83]

Tumors of Vascular and Lymphatic Tissue

Lymphangiosarcoma

Lymphangiosarcomas are rare tumors, which are seen in young dogs and cats and arise from lymphatic endothelial cells.[27,89,90] They are usually soft, cystic-like and edematous, usually occurring in the subcutis.[27] In most cases, clinical signs are associated with extensive edema and drainage of lymph through the skin or a cystic mass. Aspiration may reveal a fluid-filled mass. Histopathologically, these tumors resemble normal endothelial cells and may be confused with hemangiosarcoma because of the vascular channels; however, red blood cells are not seen.[27] Lymphangiosarcomas tend to have a moderate-to-high metastatic potential.[91] In a single case report, a dog treated with surgery that had local recurrence had a complete response following doxorubicin chemotherapy with no evidence of recurrence or metastasis 9 months after remission.[92]

Hemangioma

Hemangioma and hemangiosarcoma (HSA) are tumors of vascular endothelial origin. Hemangioma is a benign tumor that can occur in a variety of sites, including skin, liver, spleen, kidneys, bone, tongue, and heart.[93-95] Dermal hemangiomas may be induced by ultraviolet light in short-haired dogs with poorly pigmented skin.[27] Despite their benign biologic behavior, hemangiomas can cause severe anemia secondary to tumor-associated blood loss.[93,95] In humans, hemangioma can spontaneously regress or be responsive to intralesional corticosteroids, but this has not been observed in dogs.[27] Complete surgical resection is usually curative.

Hemangiosarcoma

Hemangiosarcomas are highly malignant tumors. The most common primary sites in cats and dogs involve visceral organs, especially the spleen, right atrial appendage, and liver.[24] Other primary sites include the skin, pericardium, lung, kidneys, oral cavity, muscle, bone, genitourinary tract, and peritoneum and retroperitoneum.[24,96] Primary dermal HSA has a predilection for light-haired or poorly pigmented skin on the ventral abdomen and preputial region of dogs, particularly those confined to the dermis, and solar radiation has been proposed as a cause of dermal HSA in dogs.[97]

Canine cutaneous HSAs are clinically staged according to depth of involvement with stage I tumors confined to the dermis, stage II tumors extending into subcutaneous tissue, and stage III HSAs involving the underlying muscle.[97] Stage II and III dermal HSAs are

typically large, poorly circumscribed, and have a bruised appearance which can be mistaken for a traumatic hematoma. The recommended treatment is wide surgical resection. The role of adjuvant therapies is unknown but, based on results of chemotherapy in dogs with splenic HSA,[98] doxorubicin-based chemotherapy protocols should be considered for dogs with nonmetastatic stage II and III cutaneous HSA.

Clinical staging provides prognostic information on the success of local treatment, metastatic potential, and survival time. Dogs with stage I dermal HSA have a complete surgical resection rate of 78%, 30% metastatic rate with all metastases occurring in distant dermal sites, and a median survival time of 780 days.[97] In contrast, dogs with stage II and III hypodermal HSA have a complete surgical resection rate of only 23%, principally because these tumors are larger and less well circumscribed, 60% metastatic to lungs, regional lymph nodes and distant dermal sites, and a significantly lower median survival time of 172 to 307 days (Figure 20-4).[97]

Feline cutaneous HSAs are usually solitary tumors in older cats, with a mean age of 11.5 to 12.5 years and males possibly being overrepresented.[99,100] Unlike cutaneous hemangiomas, which have no site predilection, cutaneous HSAs occur primarily in poorly pigmented skin, particularly the skin of the pinna, head, and ventral abdomen and subcutaneous tissue of the inguinal region.[99-101] Local tumor control is poor following surgical resection with local recurrence reported in 50% to 80% of cases at a median of 420 days postoperatively.[99-101] Metastasis has been reported but occurs less frequently than in dogs with dermal and hypodermal HSA.[99-101] The median survival time of greater than 1460 days for cats treated with wide surgical resection is significantly better than the median survival time of 60 days reported for untreated cats with cutaneous HSA.[100]

Tumors of Synovial Tissue

Synovial cell sarcoma

Synovial cell sarcoma is a malignant tumor arising from synoviocytes of the joint capsule and tendon sheath. There are two types of synoviocytes: type A synoviocytes are phagocytic and resemble macrophages, and type B synoviocytes are fibroblastic.[102] Synovial cell sarcomas have been classified as monophasic or biphasic depending on the proportion of malignant epithelial (synovioblastic) and mesenchymal (fibroblastic) cells within the tumor. However, this classification scheme was adopted from human medicine and is probably not applicable in small animals because of the rarity of epithelial cells in canine synovial cell sarcomas.[102] To add further confusion to the nomenclature, synovial cell sarcoma and histiocytic sarcoma are often considered as different types of joint tumors, but both may originate from synovial cells with synovial cell sarcomas
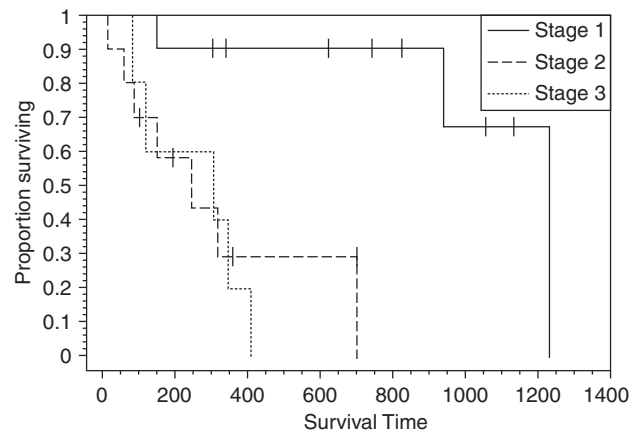
Figure 20-4

Kaplan-Meier survival curve for dogs with stage I (cutaneous), II (subcutaneous), and III (muscle) hemangiosarcoma. *(Reprinted with permission from H. Ward, L. E. Fox, M. B. Calderwood-Mays, et al., "Cutaneous hemangiosarcoma in 25 dogs: a retrospective study," J Vet Intern Med 8:345, 1994.)*

arising from type B synoviocytes and histiocytic sarcomas arising from type A synoviocytes. Expression of CD18 does not differentiate between the macrophages (or type A synoviocytes) and dendritic antigen-presenting cell lineage of histiocytic cells (PF Moore, personal communication). Immunohistochemical evidence, however, indicates that periarticular histiocytic sarcomas do arise from subsynovial dendritic antigen-presenting cells and not type A synoviocytes (P. F. Moore, unpublished data).

The diagnosis of synovial cell sarcoma is controversial, and immunohistochemistry is recommended to differentiate synovial cell sarcoma from other types of joint tumors.[12,103,104] The immunohistochemical panel should include vimentin and cytokeratin for synovial cell sarcoma, CD18 for histiocytic sarcoma, and actin for malignant fibrous histiocytoma.[103] However, other investigators have questioned the value of immunohistochemistry in the diagnosis of synovial cell sarcoma.[104] Synovial cell sarcomas are histologically graded from I to III based on criteria such as nuclear pleomorphism, mitotic figures, and necrosis, and this grading scheme provides prognostic information.[12]

Synovial cell sarcomas are locally aggressive with a moderate-to-high metastatic potential depending on histologic grade.[12] Up to 32% of dogs with synovial cell sarcomas have evidence of metastatic disease at diagnosis and 54% by the time of euthanasia.[10,12] Regional lymph nodes and lungs are the most common metastatic sites.[12] Synovial cell sarcomas have a greater metastatic potential and a higher incidence of lymph node metastasis compared to typical soft tissue sarcomas. Synovial cell sarcomas are rare in cats, but feline and

canine synovial cell sarcomas are similar in terms of histologic appearance, biologic behavior, and distribution of metastatic lesions.[105]

In dogs, synovial cell sarcoma usually occurs in large-breed dogs, with a predisposition for flat-coated and golden retrievers. Middle-aged dogs are most commonly affected, and there is no sex predilection. Synovial cell sarcomas usually involve the larger joints, particularly the stifle, elbow, and shoulder, but any joint can be affected. Lameness is the most common presenting complaint and can be confused with other orthopedic conditions.[12] Radiographic features of synovial cell sarcomas in dogs include periarticular soft tissue swelling and bone invasion, ranging from an ill-defined periosteal reaction to multifocal punctate osteolytic lesions, involving bones either side of the joint.[12,102] Bone involvement is rare in cats.[105] Limb amputation is recommended for treatment of the local tumor as local tumor recurrence is significantly lower compared to marginal or wide resection.[12,104] Local tumor recurrence following limb amputation, known as stump recurrence, occurs at a relatively higher rate than other types of tumors for which limb amputation is commonly performed.[12] Hence, the level of amputation should be as high as possible to minimize the risk of local tumor recurrence, such as forequarter amputation for synovial cell sarcomas of the thoracic limb and coxofemoral disarticulation or hemipelvectomy for synovial cell sarcomas of the pelvic limb. The role of adjuvant therapy is unknown, but synovial cell sarcomas in humans are more responsive to chemotherapy agents such as anthracyclines and ifosfamide than many other types of soft tissue sarcomas.[35] Doxorubicin-based chemotherapy protocols may be warranted in dogs with nonmetastatic grade III synovial cell sarcomas.[12,106]

Prognostic factors in dogs with synovial cell sarcoma include clinical stage, histologic grade, and extent of surgical treatment. Dogs with evidence of lymph node or lung metastasis at diagnosis have a median survival time of less than 6 months compared to 36 months or greater if there is no evidence of metastasis.[12] The median survival time for dogs treated with limb amputation is 850 days, which is significantly better than the 455 days reported following marginal resection.[104] Lastly, the median survival time for dogs with grade III synovial cell sarcomas is 7 months and significantly worse than either grade I or II synovial cell sarcomas, with median survival times greater than 48 months and 36 months, respectively (Figure 20-5).[12] In one study comparing canine joint tumors, the metastatic rate and mean survival time for dogs with synovial cell sarcoma was 25% and 31.8 months, 0% and 30.7 months for dogs with synovial myxoma, 91% and 5.3 months for histiocytic sarcoma, and 100% and 3.5 months for dogs with other types of periarticular sarcomas.[103]
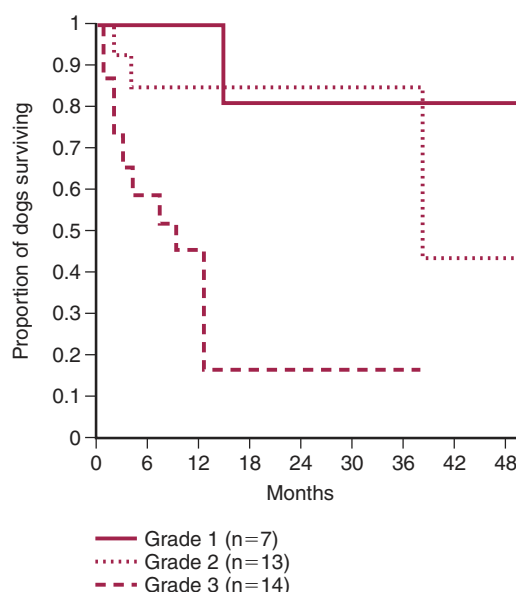
Figure 20-5

Kaplan-Meier survival curve for dogs with synovial cell sarcoma based on histologic grade. *(Reprinted with permission from D. M. Vail, B. E. Powers, D. M. Getzy, et al., "Evaluation of prognostic factors for dogs with synovial cell sarcoma: 36 cases (1986–1991)," J Am Vet Med Assoc 205:1300, 1994.)*

Tumors of Uncertain Histiogenesis

Myxosarcoma

Myxosarcomas are neoplasms of fibroblast origin with abundant myxoid matrix of mucopolysaccharides. These rare tumors occur in middle age or older dogs and cats. The majority are subcutaneous tumors of the trunk or limbs,[27] but there are reports of myxosarcomas arising from the heart, eye, and brain.[107-109] These tumors tend to be infiltrative growths with ill-defined margins.[27]

Malignant mesenchymoma

Malignant mesenchymomas are rare soft tissue sarcomas comprising a fibrous component with two or more different varieties of other types of sarcoma.[24] Malignant mesenchymoma has been reported in the lungs, thoracic wall, liver, spleen, kidney, digits, and soft tissue.[37,110-114] They have a slow rate of growth and can grow very large. Metastasis has been reported.[111,114] The outcome for dogs with splenic mesenchymoma is better than other types of splenic sarcomas, with a median survival time of 12 months and a 1-year survival rate of 50%.[37]

HISTORY AND CLINICAL SIGNS

Soft tissue sarcomas generally present as a slowly growing mass anywhere in the body. Rapid tumor growth,

intratumoral hemorrhage, or necrosis can be seen in some cases. Symptoms are directly related to site of involvement and tumor invasiveness. However, one exception is tumor hypoglycemia, which has been reported in dogs with smooth muscle tumors.[72,115] There is marked variability in the physical features of soft tissue sarcoma, but they are generally firm and adherent (fixed) to skin, muscle, or bone. Often soft tissue sarcomas can be soft and lobulated, mimicking lipomas.

Intra-abdominal tumors often compress the gastrointestinal tract and patients may present with vomiting, diarrhea, and melena. A mass may be palpable, and weight loss and anorexia is common. Leiomyosarcomas are seen most commonly in the gastrointestinal tract, spleen, and urogenital tract. Gastrointestinal leiomyosarcomas generally result in intestinal obstruction and may cause intestinal perforation leading to peritonitis.[74] Rhabdomyosarcoma, seen in young dogs and involving the bladder, presents with signs of hematuria, dysuria, and hypertropic osteopathy.[116,117]

Peripheral nerve sheath tumors, involving the brachial or lumbosacral plexus, usually result in pain, lameness, muscle atrophy, and eventual paralysis. These tumors generally are located axially and are difficult to palpate, making early diagnosis difficult, as patients do not present with clinical signs until the tumors are quite large and there is significant lameness and muscle atrophy.

DIAGNOSTIC TECHNIQUES AND WORKUP

Fine-needle aspirates are recommended to exclude other differentials, for example abscesses, cysts, or mast cell tumors. However, cytologic evaluation of fine-needle aspirates is usually not sufficient for definitive diagnosis of soft tissue sarcomas as false-negative results are relatively common because of difficulties in differentiating reactive fibrous tissue from benign and malignant sarcomas, unrepresentative samples as sarcomas have variable degrees of necrosis, and sarcomas do not exfoliate cells as readily or in the same number as epithelial and round cell tumors.[24] In one study in which fine-needle aspirates were performed on soft tissue sarcomas from 40 dogs, 15% of dogs were incorrectly diagnosed and a further 23% were nondiagnostic.[118] Biopsy methods for definitive preoperative diagnosis of soft tissue sarcomas include needle-core, punch, incisional, or excisional biopsies. The biopsy should be planned and positioned so that the biopsy tract can be included in the curative-intent treatment, whether it be surgery or radiation therapy, without increasing the surgical dose or size of the radiation field. Excisional biopsies are not recommended as they are rarely curative and the subsequent surgery required to achieve complete histologic margins is often more

aggressive than surgery following core or incisional biopsies, resulting in additional morbidity and treatment costs. Furthermore, multiple attempts at resection, including excisional biopsy, prior to definitive therapy has a negative impact on survival time in dogs with soft tissue sarcomas.[119]

The tests performed for workup and clinical staging can be dependent on the type of soft tissue sarcoma, especially if atypical (e.g., HSA, histiocytic sarcoma, lymphangiosarcoma, and synovial cell sarcoma), but usually involves routine hematologic and serum biochemical blood tests, three-view thoracic radiographs, and regional imaging of the soft tissue sarcoma. Blood tests are usually within the normal reference range for most dogs with soft tissue sarcomas; however, there are some notable exceptions. Hematologic abnormalities, such as anemia and thrombocytopenia, are relatively common in dogs with disseminated histiocytic sarcoma and HSA.[24,43,44] Hypoglycemia has been reported in dogs with intra-abdominal leiomyomas and leiomyosarcomas.[68,71,72,115] Excessive production of insulin-like growth factor II has been implicated as the cause of hypoglycemia in humans with mesenchymal tumors, and this has also been demonstrated in one dog with a gastric leiomyoma.[120-122]

Imaging studies of the local tumor may be required for planning of the surgical approach or radiation therapy if the tumor is fixed to underlying structures or located in an area that may make definitive treatment difficult, such as the pelvic region. Three-dimensional imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI) scans, are particularly useful for staging local disease (see Figure 20-2).[123] Other imaging modalities for staging of the local tumor include survey radiographs, ultrasonography, angiography, and nuclear scintigraphy.

Diagnostic tests for staging of metastatic disease include thoracic radiographs, abdominal ultrasonography or advanced imaging, and fine-needle aspirates or biopsy of the regional lymph nodes. Three-view thoracic radiographs should be performed prior to definitive treatment, as the lungs are the most common metastatic site for typical soft tissue sarcomas.[16] Lymph node metastasis is uncommon with typical soft tissue sarcomas. Fine-needle aspiration or biopsy of regional lymph nodes should be performed in dogs with clinically abnormal lymph nodes or atypical soft tissue sarcomas with a high rate of metastasis to regional lymph nodes, such as HSA, histiocytic sarcoma, lymphangiosarcoma, synovial cell sarcoma, leiomyosarcoma, and possibly rhabdomyosarcoma.[12,32,33,68,74-76,97] Abdominal imaging is recommended for assessment of metastasis to intra-abdominal organs, especially the lymph nodes, spleen, and liver, in animals with suspected intra-abdominal neoplasia (e.g., gastrointestinal leiomyosarcoma or splenic sarcoma) or pelvic limb soft tissue sarcoma.

Box 20-2	Modified Staging System for Canine Soft Tissue Sarcomas

Modified staging system for soft tissue sarcomas

Primary Tumor (T)

T1   Tumor ≤5 cm in diameter at greatest dimension
T1a   Superficial tumor
T1b   Deep tumor
T2   Tumor >5 cm in diameter at greatest dimension
T2a   Superficial tumor
T2b   Deep tumor

Regional lymph nodes (N)

N0   No regional lymph node metastasis
N1   Regional lymph node metastasis

Distant metastasis (M)

M0   No distant metastasis
M1   Distant metastasis

Stage grouping	Tumor (T)	Nodes (N)	Metastasis (M)	Grade
I	Any T	N0	M0	I–II
II	T1a-1b, T2a	N0	M0	III
III	T2b	N0	M0	III
IV	Any T	N1	Any M	I–III
	Any T	Any N	M1	I–III

A modified staging system has been described for soft tissue sarcomas in dogs.[24] The American Joint Committee on Cancer (AJCC) staging system currently used in humans with soft tissue sarcomas has been substantially modified from the original staging system, on which the modified animal staging system is based. The most important changes to the AJCC staging system are categorization of local disease with less emphasis on tumor size, which is an arbitrary assignment, and greater emphasis on depth of invasion.[35,124] A superficial tumor is defined as a soft tissue sarcoma located above the superficial fascia, which does not invade the fascia, whereas a deep tumor is located deep to the superficial fascia, invades the fascia, or both.[124] Based on the current AJCC staging system, an updated modified staging system for animals is summarized in Box 20-2. The stage grouping takes into account both clinical staging criteria (TNM staging system) and histologic grade. The original and updated staging systems for animals with soft tissue sarcomas have not been investigated, and the prognostic significance of either of these staging systems is unknown.

TREATMENT

There are more than 20 different histologic subtypes of soft tissue sarcomas described in dogs, but the vast majority of these subtypes have a similar biologic behavior, which is characterized by local aggression and a low-to-moderate metastatic potential depending on histologic grade.[16] Local tumor control is the most important consideration in the management of soft tissue sarcomas because of their locally aggressive behavior. As such, surgical resection is the principal treatment for dogs with soft tissue sarcomas. Radiation therapy also plays an important role in local tumor control, especially for incompletely resected and unresectable soft tissue sarcomas. However, definitive treatment options depend on histologic subtype (especially for atypical soft tissue sarcomas such as HSA, histiocytic sarcoma, and lymphangiosarcoma), tumor location, clinical stage, histologic grade, and completeness of surgical margins.[24,125] A strategy for managing dogs with typical soft tissue sarcomas is presented in Figure 20-6.

Surgery

Soft tissue sarcomas are locally aggressive tumors that grow along paths of least resistance and invade surrounding tissue resulting in the formation of a pseudocapsule of compressed viable tumor cells.[24] The pseudocapsule gives the false impression of a well-encapsulated tumor. However, surgical removal of the encapsulated mass without adequate margins will result in incomplete resection and a high risk of local tumor
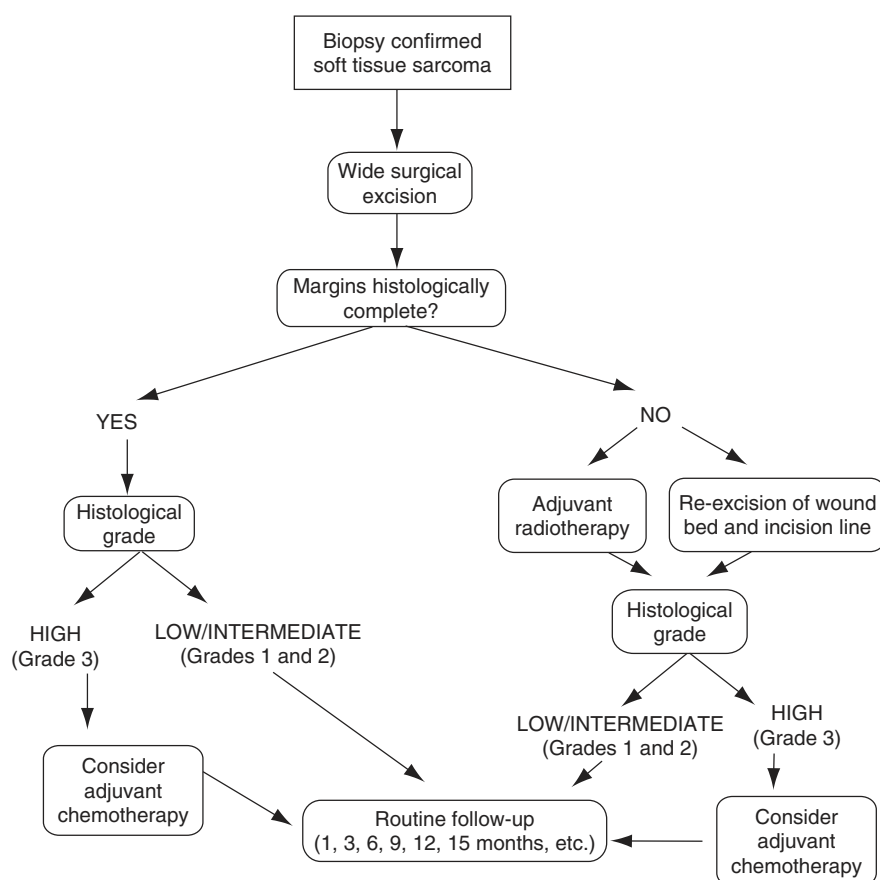
Figure 20-6
Suggested treatment plan for dogs with nonmetastatic soft tissue sarcoma.

recurrence.[16] The minimum recommended margins for surgical resection are 3 cm lateral to the tumor and one fascial layer deep to the tumor (Figure 20-7).[125,126] Biopsy tracts and any areas of fixation, including bone and fascia, should be resected *en bloc* with the tumor using the recommended surgical margins. Radical surgery, such as limb amputation or hemipelvectomy, may be required to achieve adequate surgical margins and local tumor control.

The resected tumor should be pinned out to the original dimensions to prevent shrinkage during formalin fixation (Figure 20-8),[127] the lateral and deep margins should be inked to aid in histologic identification of surgical margins, and any areas of concern should be tagged with suture material, inked in a different color, or submitted separately for specific histologic assessment. Surgical margins and tumor grade are important in determining the need and type of further treatment. For instance, tumor grade is important in deciding whether a soft tissue sarcoma resected with complete but close surgical margins (i.e., 1 to 3 mm) will require

further local treatment as surgical margins may be adequate with a low-grade soft tissue sarcoma but not for a high-grade soft tissue sarcoma.[125,126]

The first surgery provides the best opportunity for local tumor control as the management of incompletely resected tumors increases patient morbidity and treatment costs, increases the risk of further local tumor recurrences, and decreases survival time.[119,125,128-132] If the tumor has been incompletely resected, the surgical scar can be managed with either a second surgery or radiation therapy. The entire surgical wound is contaminated and considered neoplastic.[125] As such, wide resection of the surgical scar should be performed with the same margins recommended for a primary soft tissue sarcoma, 3 cm lateral to the tumor and one fascial layer deep to the tumor.[123,125,130,131] Similarly, if radiation therapy is used to control local disease following incomplete resection, the entire surgical scar should be included in the radiation field.[130,131] Surgery is preferred to radiation therapy for management of incompletely resected soft tissue sarcomas as, in
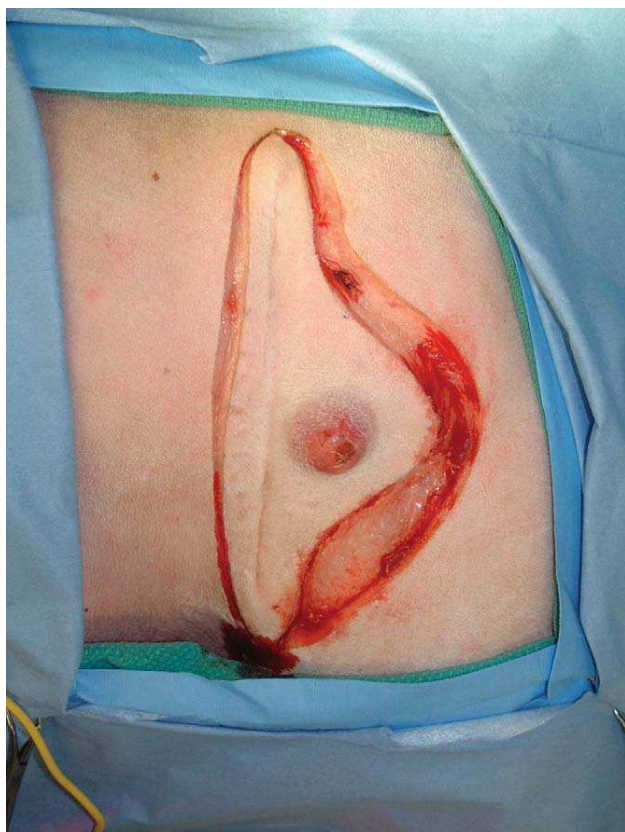
Figure 20-7

Intraoperative image of a dog with a recurrent soft tissue sarcoma. The soft tissue sarcoma is being resected with 3 cm margins around the recurrent tumor and previous scar, as both are considered contaminated, and deep margins of one fascial layer.

Figure 20-8

Following surgical resection, the lateral and deep margins should be inked (yellow ink was used in this case), to assist in identification of the margins histologically, and pinned out on cardboard to prevent sample shrinkage during formalin fixation.

Figure 20-9

Planned marginal resection of a soft tissue sarcoma in a dog. Marginal resection followed by full course postoperative radiation therapy provides excellent local tumor control and preserves both the limb and limb function. Radiation therapy should not involve the limb circumferentially to preserve both lymphatic and venous drainage of the distal extremity.

humans, local tumor control is better with repeat surgical resection than adjunctive radiation therapy alone.[130,131]

Surgery and Radiation Therapy

Radiation therapy can be used as an adjunct to surgery following either planned marginal resection or unplanned incomplete resection. Marginal surgical resection combined with full-course postoperative radiation therapy is an attractive alternative to limb amputation for extremity soft tissue sarcoma (Figure 20-9). This multimodality approach requires additional planning but preserves the limb and limb function. Surgery involves complete removal of all grossly visible tumor and then marking the lateral, proximal, and distal extents of the surgical field with radiopaque clips to assist in planning of radiation therapy. Migration of the radiopaque clips has been reported but does not significantly influence the planned radiation field.[133]

Radiation therapy should be started a minimum of 7 days postoperatively to minimize the risk of radiation-induced complications with the surgical wound, such as delayed healing and dehiscence.[134] Full-course fractionated protocols are recommended with reported schedules including 3.0 to 4.2 Gy fractions on a
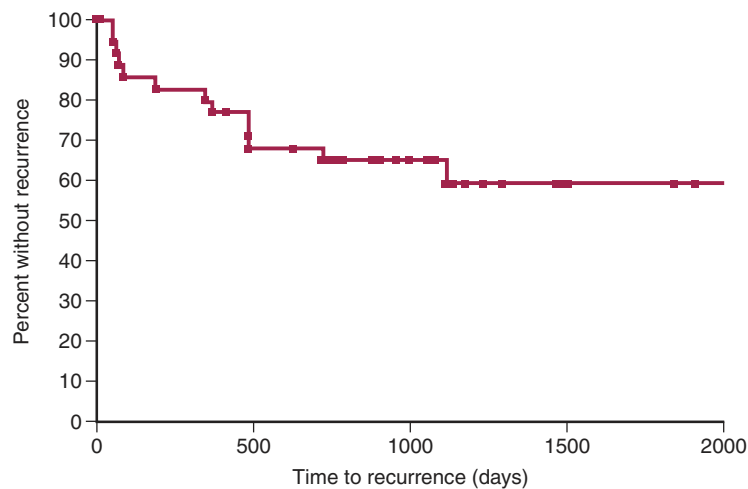
Figure 20-10

Kaplan-Meier curve for time to local tumor recurrence in 35 dogs treated with surgery (incomplete resection) and postoperative radiation therapy. *(Reprinted with permission from L. J. Forrest, R. Chun, W. M. Adams, et al., "Postoperative radiotherapy for canine soft tissue sarcoma," J Vet Intern Med 14:578, 2000.)*

Monday-to-Friday or Monday-Wednesday-Friday schedule to a total dose of 42 to 63 Gy.[135,136] The optimal fractionated and total doses for canine soft tissue sarcoma have not been determined, but cumulative doses >50 Gy are recommended and local tumor control is better with higher cumulative doses.[135] Acute side effects of radiation therapy, such as moist desquamation, are relatively mild and transient.[136]

Local tumor control and survival time are usually excellent when incompletely resected soft tissue sarcomas are treated with postoperative radiation therapy. The median time to local recurrence is 700 days to greater than 798 days with local tumor recurrence reported in 5% to 30% of dogs by 1 year and 16% to 60% of dogs in the long-term (Figure 20-10).[119,135-139] The overall median survival time for incompletely resected nonoral soft tissue sarcomas treated with postoperative radiation therapy is 2270 days with 2-, 4-, and 5-year survival rates of 87%, 81%, and 76%, respectively (Figure 20-11).[135,136]

Radiation Therapy

Radiation therapy can be employed along with surgery in the treatment of soft tissue sarcomas with a curative intent, either preoperatively or postoperatively, or as a sole treatment for pain palliation. Use of radiotherapy alone as a single modality treatment using doses of 50 Gy resulted in 1-year tumor control rates of 50% that dropped to 33% at 2 years.[140] Measurable and palpable soft tissue sarcomas are resistant to long-term control with conventional doses of irradiation alone (40 to 48 Gy).[141,142] Although one study reported a 30% complete response rate with radiation therapy alone,[143] these tumors do not rapidly regress after radiation, or if there is significant tumor shrinkage, it is not a durable response. As a single modality, radiotherapy is generally considered palliative with control defined as a slowly regressing or stable-in-size tumor mass.

Inadequate durable tumor control using radiotherapy as a single modality resulted in the development of combination therapies using radiation therapy with hyperthermia and surgery, yielding greater control rates.[15,135,136,143-147] Current results of therapeutic clinical studies in dogs demonstrate that radiation therapy is the best treatment for incompletely resected soft tissue sarcomas.[15,135,136,147] More recent studies using megavoltage irradiation (45 to 57 Gy) have yielded 1-year control rates of 48% to 67%.[140,144]

Although higher doses of irradiation will have higher control rates, the chances of normal tissue complications also increase. In some studies, hyperthermia combined with irradiation showed promise for improved control versus irradiation alone and may also decrease the time to recurrence.[143-145] The median duration of local control with radiation therapy plus hyperthermia is 750 days and significantly greater than the 350 days with radiation therapy alone. Difficulty in homogenous heating of large tumors limits the routine use of hyperthermia for treating soft tissue sarcoma in conjunction with radiation. The addition of whole-body hyperthermia does not improve response rate when compared to radiation therapy and local hyperthermia and may increase the risk for metastasis.[146]

As discussed previously, the combination of radiation and surgery provides long-term local control. Postoperative irradiation may be utilized if surgical
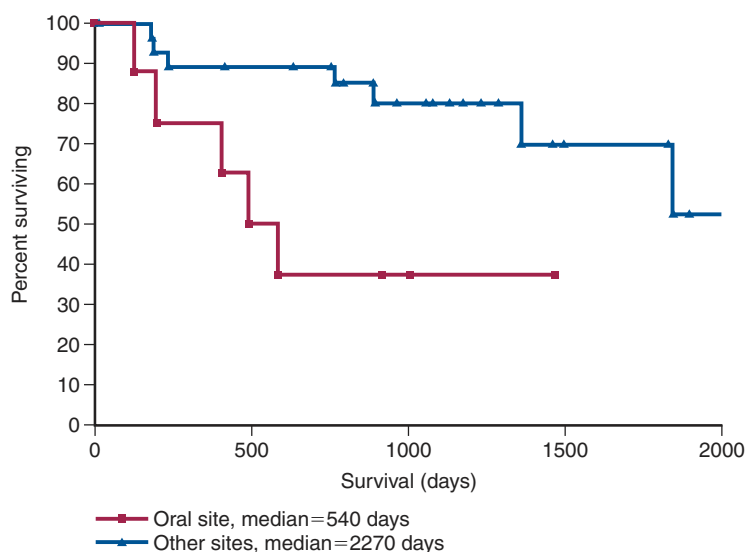
Figure 20-11

Kaplan-Meier survival curve of 35 dogs treated with surgery (incomplete resection) and postoperative radiation therapy after soft tissue sarcomas of oral sites were separated from other locations. *(Reprinted with permission from L. J. Forrest, R. Chun, W. M. Adams, et al., "Postoperative radiotherapy for canine soft tissue sarcoma," J Vet Intern Med 14:578, 2000.)*

removal is incomplete and further surgery is not feasible. Irradiation of microscopic tumors, following excision, is generally superior to radiation of measurable tumors. Control rates for adjuvant radiation after histologically incomplete resection of soft tissue sarcomas are 80% to 95% at 1 year and 72% to 91% at 2 years, with an expected 3-year and 5-year survival rate of 68% and 76%, respectively.[135,136] Although not statistically significant, dogs with hemangioma-pericytoma (HPC) had a 3-year survival rate of 92% in one retrospective study.[135] Preoperative radiation therapy is becoming commonplace in veterinary oncology. The rationale and advantage to administering radiation therapy prior to surgery are that (1) the radiation field is smaller because, following surgery, the entire surgical site must be included in the field and this may contribute to local toxicity; (2) the inactivation of a large number of peripheral tumor cells (with reduced contamination of the surgical site); and (3) tumor reduction may make surgical resection less difficult.[15,148-150] In a study of 112 human patients with soft tissue sarcomas, it was noted that there was no significant difference between pre- or postoperative radiotherapy in terms of relapse-free survival, local control, or overall survival. However, wound complications were significantly more frequent in preoperative radiotherapy patients.[150] A review continues to support these findings.[151] Lower doses of preoperative radiotherapy (less than 50 Gy) are used to reduce the risk of surgical complications. The therapeutic goal of preoperative radiotherapy is to eradicate the

microscopic tumor cells at the peripheral margins. Generally, preoperative radiotherapy is reserved for initially inoperable tumors as an alternative to radical surgery, which provides a better cosmetic outcome.

Chemotherapy

The role of chemotherapy in the management of dogs with soft tissue sarcoma is unknown. The metastatic rate for cutaneous soft tissue sarcoma is grade dependent and varies from less than 15% for grade I and II soft tissue sarcoma to 41% for grade III soft tissue sarcoma.[16] Metastasis often occurs late in the course of disease, with a median time to metastasis of 365 days,[16] and this may minimize the beneficial effects of postoperative chemotherapy on the development of metastatic disease. However, there are clinical situations in which postoperative chemotherapy should be considered, including dogs with grade III soft tissue sarcoma, metastatic disease, intra-abdominal soft tissue sarcoma (e.g., leiomyosarcoma and splenic sarcoma), and histologic subtypes with a higher rate of metastasis, such as histiocytic sarcoma, hypodermal HSA (stage II or III), synovial cell sarcoma, rhabdomyosarcoma, and lymphangiosarcoma.[24,152]

Doxorubicin-based protocols, either alone or in combination with cyclophosphamide, have shown the most promise in dogs with soft tissue sarcoma with an overall response rate of 23%.[153] The need to combine cyclophosphamide with doxorubicin is debatable as single-agent doxorubicin has been shown to be equally as
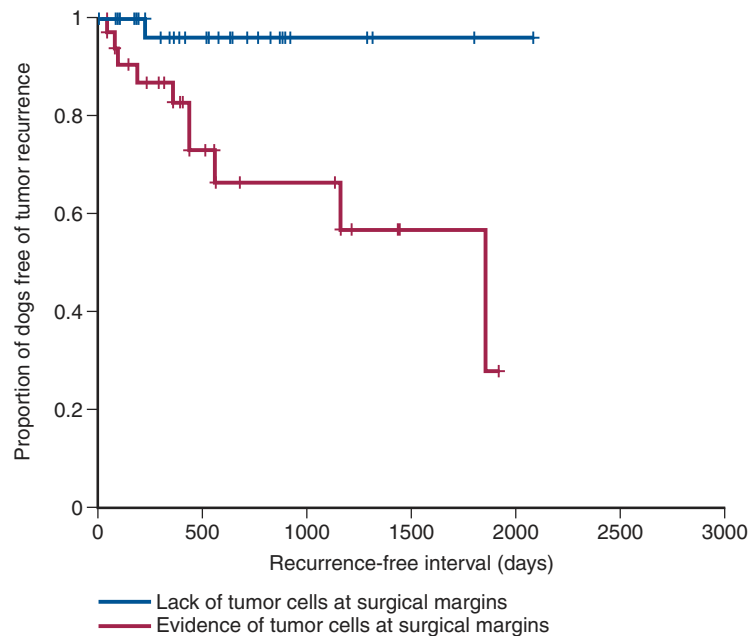
Figure 20-12

Kaplan-Meier curve for disease-free interval in 39 dogs with complete surgical removal of soft tissue sarcomas and 36 dogs with incomplete surgical margins. *(Reprinted with permission from C. A. Kuntz, W. S. Dernell, B. E. Powers, et al., "Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986–1996)," J Am Vet Med Assoc 21:1147, 1997.)*

effective in the management of dogs with HSA as doxorubicin combined with either cyclophosphamide or cyclophosphamide and vincristine.[98] Mitoxantrone, a chemotherapeutic drug related to doxorubicin, has a variable effect against soft tissue sarcoma in dogs with response rate varying from 0% in six dogs to 33% in 12 dogs.[154,155] Ifosfamide has also shown some potential with a complete response rate of 15% in 13 dogs with solid sarcomas of the skin, bladder, and spleen.[156] Doxorubicin and ifosfamide are the most effective single agents in the management of soft tissue sarcoma in humans, but response rates are less than 30% and meta-analyses show single- and multiple-agent chemotherapy protocols do not significantly increase overall survival times compared to surgery alone.[157,158] Furthermore, in dogs, the combination of doxorubicin and ifosfamide does not appear to be more efficacious than doxorubicin alone.[159]

In one study, postoperative doxorubicin did not influence either the metastasis-free interval or overall survival time in dogs with grade III soft tissue sarcoma, but there was a significant decrease in the rate of local tumor recurrence in dogs with incompletely resected tumors.[160] Local release of cisplatin from a biodegradable polymer implanted into the surgical bed of incompletely resected soft tissue sarcomas may also decrease the risk of local tumor recurrence, with a 31% rate of local tumor recurrence in 32 dogs at a median of greater than 640 days

postoperatively.[161] Postoperative systemic chemotherapy has also been shown to significantly improve disease-free survival times, but not overall survival time, in humans with soft tissue sarcomas, regardless of histologic grade.[157,158]

PROGNOSIS

The prognosis for dogs with soft tissue sarcoma is good. Local tumor control is often the most challenging aspect of managing soft tissue sarcomas.[16,118] Local tumor recurrence rates following either surgery alone or surgery and radiation therapy varies from 7% to 32%.[118,119,137,162] Poor prognostic factors for local tumor recurrence include large tumor size, incomplete surgical margins, and high histologic tumor grade.[16] In one study of 75 dogs, the local tumor recurrence rate following incomplete resection was 28% and 11 times more likely than soft tissue sarcomas resected with complete margins (Figure 20-12).[16] Tumor size has been reported to have a negative effect on local tumor control,[16] but tumor size probably influences the ability to achieve complete resection rather than having a direct effect on local tumor recurrence. Furthermore, tumor size has not been identified as a prognostic factor in other studies[119,137,162] and tumor size does not influence local

tumor control in dogs treated with surgery and adjuvant radiation therapy or radiation therapy alone.[140,144,162,163] Management of recurrent soft tissue sarcomas is usually more difficult than primary tumors, which emphasizes the need for an aggressive approach initially. Curative-intent treatment of recurrent soft tissue sarcomas often requires a more aggressive approach resulting in increased treatment-related morbidity, while the disease-free interval is shorter, the metastatic rate higher, and survival times are decreased in comparison to dogs with primary tumors.[119,125,128-132] Local tumor recurrence is still possible after either complete resection or incomplete resection combined with adjunctive radiation therapy.[16,135,136] Consequently, examination of the treatment site is recommended at regular intervals, such as monthly for the first 3 months, then every 3 months for the first 12 months, and every 6 months thereafter.[125] The median time to local tumor recurrence was 368 days in one report of 75 dogs with soft tissue sarcomas, which emphasizes the need for long-term follow-up in these cases.[16]

The metastatic rate in dogs with soft tissue sarcoma varies from 8% to 17% with a median time to metastasis of 365 days.[16,135,136] Factors that increase the risk of metastatic disease include histologic grade, number of mitotic figures, percentage of tumor necrosis, and local tumor recurrence. As mentioned previously, the metastatic rate for dogs with grade I or II soft tissue sarcomas is less than 15% compared to 41% for grade III soft tissue sarcomas.[16] Metastasis is five times more likely when tumors have a mitotic rate ≥20 mitotic figures per 10 high power fields compared to <20 mitotic figures per 10 high power fields.[16]

The median survival time for dogs with soft tissue sarcoma ranges from 1416 days following surgery alone to 2270 days with surgery and adjunctive radiation therapy.[16,135,136] Overall, up to 33% of dogs eventually die of tumor-related causes.[16] Tumor-related deaths in dogs with soft tissue sarcoma is 2.8 times more likely with >10% tumor necrosis and 2.6 times more likely with mitotic rate ≥20 mitotic figures per 10 high power fields.[16] The median survival times for dogs with ≤10, 10 to 19, and ≥20 mitotic figures per 10 high power fields are 1444 days, 532 days, and 236 days, respectively (Figure 20-13).[16] The prognosis for specific subtypes of soft tissue sarcoma is discussed separately earlier in this chapter under Specific Tumor Types.

FELINE SARCOMAS AND VACCINE-ASSOCIATED SARCOMAS

Epidemiology and Risk Factors

The following events are linked to the development of postvaccinal sarcomas in the cat. The prevalence of feline rabies led to the enactment of a law requiring rabies vaccinations for cats in Pennsylvania in 1987.[164] In addition, two changes in vaccines occurred in the mid-1980s: development of a killed rabies vaccine licensed for subcutaneous administration and a killed vaccine
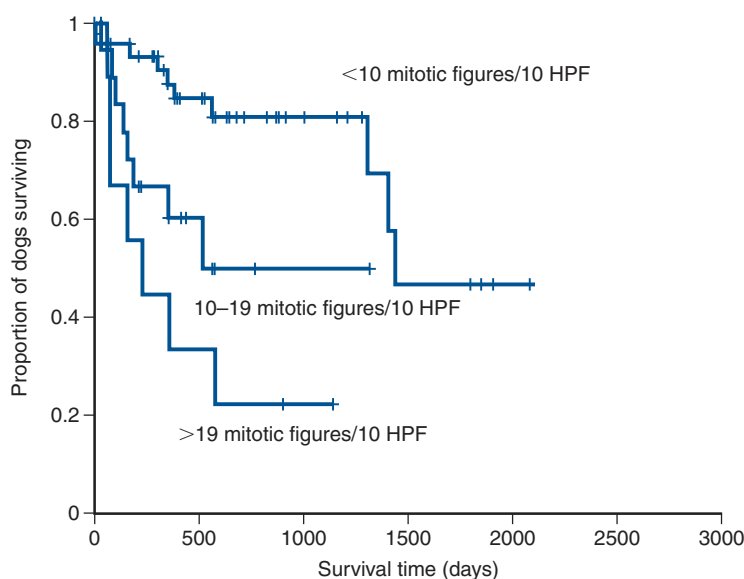
Figure 20-13

Kaplan-Meier survival curve for 75 dogs with soft tissue sarcomas based on mitotic rates. *(Reprinted with permission from C. A. Kuntz, W. S. Dernell, B. E. Powers, et al., "Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986–1996)," J Am Vet Med Assoc 21:1147, 1997.)*

for feline leukemia virus (FeLV).[165] Two pathologists, M. J. Hendrick and M. H. Goldschmidt, and their colleagues at the University of Pennsylvania School of Veterinary Medicine were the first to recognize the increased incidence of reactions and formation of sarcomas at sites of rabies vaccinations.[166,167] Epidemiologic studies have shown a strong association between the administration of inactivated feline vaccines, such as FeLV and rabies, and subsequent development of soft tissue sarcoma development at these vaccine sites.[165-169] Additionally, some authors report reaction to vaccines was additive and increasing the likelihood of sarcoma development with multiple vaccines given at the same site simultaneously.[169] The development of soft tissue sarcoma at sites of vaccine administration of FeLV or rabies is believed by some to be as high as 1/1000,[170,171] while others report a prevalence between 1 to 3.6/10,000 cases.[169,172,173] Vaccine-associated sarcomas are comparably more prevalent than fibrosarcomas at nonvaccine sites, with the latter accounting for 20/100,000 cases.[24]

The time to tumor development postvaccination has been reported to be 4 weeks to 10 years.[174] An association of soft tissue sarcoma development with feline panleukopenia and feline rhinotracheitis has been reported,[168,171,175] and adjuvant vaccines are more likely to cause a vaccine site reaction or develop into a soft tissue sarcoma.[174] However, two large epidemiologic studies did not provide evidence that aluminum-containing vaccines pose a greater risk.[168,169] Thus, it remains unclear whether nonadjuvant vaccines are safer.[176] A multicenter study of cats in the United States and Canada found that no single vaccine manufacturer or vaccine type had a higher or lower association with the development of soft tissue sarcoma. Additionally, vaccine practices such as needle gauge, syringe reuse, use and shaking of multidose vials, mixing vaccines in a single syringe, and syringe type have no role in the development of tumors.[176]

The hypothesis that postvaccination inflammatory reactions lead to uncontrolled fibroblast and myofibroblast proliferation and eventual tumor formation either alone or along with immunologic factors is a popular theory.[177-181] The thought that inflammation precedes tumor development is supported by histologic identification of transition zones from inflammation to sarcoma and microscopic foci of sarcoma located in areas of granulomatous inflammation.[182] A similar phenomenon of intraocular sarcoma development exists in cats after trauma or chronic uveitis.[183-185]

Growth factors regulate the cellular events involved in granulation tissue formation and wound healing. When these factors are added to fibroblast cultures, the cells develop a neoplastic phenotype. Immunohistochemical identification and localization of growth factors and their receptors are being investigated in vaccine-associate sarcomas.[179] Through immunohistochemical studies of growth factors and their receptors, Hendrick has found vaccine-associated sarcomas are immunoreactive for platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and their receptors, and transforming growth factor-β. Conversely, non-vaccine-associated fibrosarcomas are negative or faintly positive.[178] Hendrick also found that lymphocytes in vaccine-associated sarcomas are positive for PDGF, but lymphocytes in non-vaccine-associated fibrosarcomas and in normal lymph nodes are negative.[186] Regional macrophages also stain positively for PDGF receptor. Neoplastic cells that are closest to lymphocytes in these tumors have the strongest staining for PDGF receptor, which has led to a hypothesis that lymphocytes in vaccine-associated sarcomas may secrete PDGF, recruit macrophages, and lead to fibroblast proliferation.[178,187] The expression of c-jun, a proto-oncogene coding for the transcriptional protein AP-1, has also been examined in vaccine-associated sarcomas. C-jun was found to be strongly positive in vaccine-associated sarcomas and not expressed in non-vaccine-associated fibrosarcomas.[178] It appears that FeLV and the feline sarcoma virus are not involved in the pathogenesis of feline vaccine-associated sarcomas. Using immunohistochemical analysis and polymerase chain reaction techniques, FeLV was not detected in vaccine-associated sarcomas.[188]

p53 mutations have been evaluated in feline vaccine-associated sarcomas.[188,190] p53 is a tumor suppressor gene, which encodes a nuclear protein critical in the regulation of the cell cycle. Normal or wild-type p53 will increase in response to DNA damage resulting in cell arrest at the G1 interphase, allowing for DNA repair before replication or apoptosis if damage is irreparable. Cells lacking normal p53 proceed through the cell cycle unregulated, leading to aberrant clones and possible malignancy.[179] Anti-p53 antibodies have immunoreactivity in feline sarcomas and may play a role in predicting clinical outcome.[191]

Papers continue to link growth factors with development of vaccine-associated sarcomas in cats. Continued immunohistichemical probing of feline vaccine-associated sarcomas document expression of growth-regulating proteins: p53 protein, basic fibroblast growth factor-b and transforming growth factor-α.[192] Researchers have concluded that PDGF and its receptor play an important role in the *in vitro* growth of vaccine-associated sarcoma cell lines, both alone and in the presence of chemotherapeutic agents. Furthermore, they found that a signal transduction inhibitor, imatinib mesylate, inhibits the PDGF-dependent cell growth in a dose-dependent manner.[180]

Pathology

There are many similarities between the histologic subtypes and biologic behavior of soft tissue sarcomas

in cats and dogs. The three principal exceptions in cats are vaccine-associated sarcomas, virally induced multicentric fibrosarcoma, and the relative rarity of PNST, synovial cell sarcoma, and histiocytic sarcoma.[31,181] There are significant differences between vaccine-associated sarcoma and non-vaccine-associated sarcoma. Tumors that develop after vaccination are typically mesenchymal in origin and include fibrosarcoma, rhabdomyosarcoma, malignant fibrous histiocytoma, undifferentiated sarcoma, and extraskeletal osteosarcoma and chondrosarcoma.[185,193,194] Vaccine-associated sarcomas have histologic features consistent with a more aggressive biologic behavior than non-vaccine-associated sarcoma, such as marked nuclear and cellular pleomorphism, increased tumor necrosis, high mitotic activity, and the presence of a peripheral inflammatory cell infiltrate consisting of lymphocytes and macrophages.[180,185,195] In a series of 100 cats with histologically confirmed and graded vaccine-associated sarcoma, the prevalence of high-grade lesions was substantially higher than that reported in dogs,[16] with 60% of cats diagnosed with grade III tumors and only 6% with grade I tumors (C. A. Kuntz, unpublished data). Microscopically, areas of transition between inflammation and tumor development are frequently observed in cats with vaccine-associated sarcoma.[163,185,196] The macrophages in these peripheral inflammatory cell infiltrates often contain a bluish gray foreign material, which has been identified as aluminum and oxygen by electron probe x-ray microanalysis.[181] Aluminum hydroxide is one of several adjuvants used in currently available feline vaccines.[181] Vaccine-associated sarcomas are histologically similar to mesenchymal tumors arising in traumatized eyes of cats, which suggests a common pathogenesis of inflammation and the development of soft tissue sarcomas in these cats.[183-185] The presence of inflammatory cells, fibroblasts, and myofibroblasts in and adjacent to vaccine-associated sarcomas supports this hypothesis.[24,197,198]

Diagnosis and Workup

The diagnostic techniques and clinical staging tests recommended for cats with suspected vaccine-associated sarcoma are similar to those described in dogs earlier in this chapter. Advanced imaging, such as contrast-enhanced CT or MRI, is recommended for local staging of the tumor, as three-dimensional imaging modalities provide essential information for proper planning of surgery or radiation therapy (Figure 20-14).[181] The volume of tumor based on contrast-enhanced CT is approximately twice the volume measured using calipers during physical examination.[178] Accurate pretreatment knowledge of the extent of disease is important, as vaccine-associated sarcomas are very invasive, frequently located in areas in which regional anatomy can
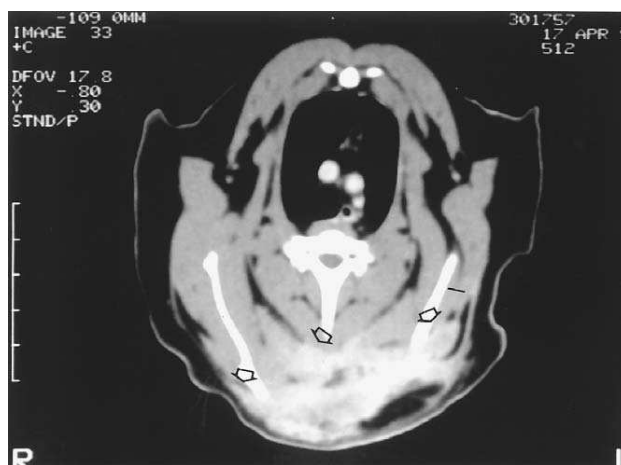
Figure 20-14

Contrast-enhanced CT image of a cat with a vaccine-associated sarcoma. Note the finger-like projections of the tumor (*arrows*).

complicate an aggressive surgical approach (e.g., interscapular area, body wall, and proximal pelvic limb), and have a high rate of local tumor recurrence, especially if incompletely resected. Excisional biopsy of a suspected vaccine-associated sarcoma is not recommended because the risk of local tumor recurrence increases and disease-free interval and survival time significantly decrease.[199,200]

Treatment

The Vaccine-Associated Feline Sarcoma Task Force has recommended that masses at vaccination sites be treated if the mass is still evident ≥3 months after vaccination, >2 cm in diameter, or are increasing in size more than 4 weeks after vaccine administration.[181] Vaccine-associated sarcomas are very invasive tumors and aggressive treatment is required, with both wide surgical resection and full-course radiation therapy, to achieve adequate local control.

Surgery

Vaccine-associated sarcomas are poorly encapsulated tumors with extension and infiltration along fascial planes.[201,202] The Vaccine-Associated Feline Sarcoma Task Force has recommended surgical resection with a minimum of 2 cm margins both lateral and deep to the tumor.[181] Marginal resection or excisional biopsy should not be attempted. The median disease-free interval and survival time are significantly decreased with marginal resection, increased number of surgical interventions, and surgery performed by nonreferral surgeons (Figure 20-15).[194,199,200] The median time to first recurrence following marginal resection is 79 days compared
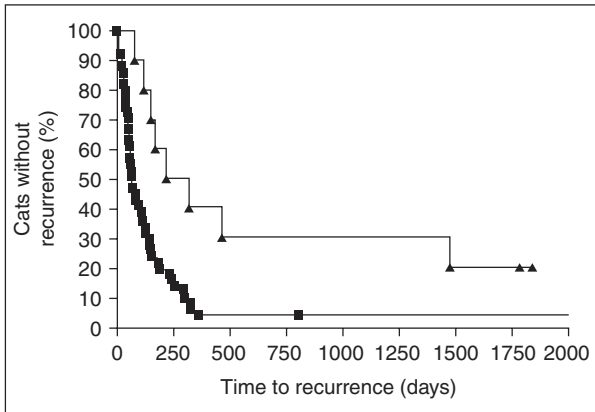
Figure 20-15

Kaplan-Meier curve for time to first local tumor recurrence in 47 cats with vaccine-associated sarcomas treated by referring veterinarians (*squares*) compared to 14 cats treated at referral institutions (*triangles*). *(Reprinted with permission from A. E. Hershey, K. U. Sorenmo, M. J. Hendrick, et al., "Prognosis for presumed feline vaccine-associated sarcoma after excision: 61 cases (1986–1996)," J Am Vet Med Assoc 216:58, 2000.)*

Figure 20-16

Aggressive resection of vaccine-associated sarcomas is required for prolonged local tumor control. In this cat, the vaccine-associated sarcoma was resected with 5 cm margins, including the dorsal spinous processes (*D*). The scapulae (*S*) were spared as the tumor could be resected with two fascial planes without involving the scapula.

to 325 to 419 days for wide resection or radical surgery.[200] In addition, the median time to first recurrence is only 66 days when the first surgery is performed at a nonreferral institution compared to 274 days at referral institutions.[200] Inadequate biopsy planning, preoperative staging, or attempts at first surgery will result in an increase in tumor margins and may make further surgical treatment impossible. The first attempt at surgical management of cats with vaccine-associated sarcomas should be performed by a referral surgeon with experience in aggressive resection, especially in the interscapular and pelvic regions, to increase the chance of a successful outcome.[178]

Similar to dogs with soft tissue sarcomas, biopsy tracts and any areas of fixation, including bone and fascia, should be resected *en bloc* with the tumor. In cats with vaccine-associated sarcoma, wide surgical resection of tumors located in the interscapular region will often involve excision of dorsal spinous processes and perhaps the dorsal aspect of the scapula for tumors (Figure 20-16), while thoracic or body wall resection is often required for truncal tumors.[199,200] Limb amputation or hemipelvectomy is usually required to achieve adequate surgical margins and local tumor control for vaccine-associated sarcoma located on the extremity.

Local tumor control is still disappointing with curative-intent surgery. Despite attempting aggressive surgical margins, complete resection is achieved in less than 50% of cats.[199,200] Furthermore, overall 1- and 2-year

disease-free intervals are only 35% and 9%, respectively.[199] Median disease-free interval and survival time are both greater than 16 months following complete histologic resection and significantly better than incompletely resected tumors. Local tumor control is improved for extremity vaccine-associated sarcomas, presumably because the lateral and deep surgical margins achieved with limb amputation are superior to other locations.[200] However, aggressive surgery is possible in other locations with good results. Chest wall and body resection, using a minimum of 3 cm margins, was well-tolerated in six cats and local tumor recurrence was not reported in any of these cats at a minimum of 12 months postoperatively.[203]

Some surgeons have advocated a more aggressive surgical approach because of the low rate of complete resection, and subsequent poor local tumor control, with attempts at curative-intent surgery using minimum margins of 2 to 3 cm. This is supported by one study of 100 cats with vaccine-associated sarcoma in various locations, including interscapular, trunk, and extremity, treated with surgery alone using 5 cm lateral margins and two fascial planes for deep margins (C. A. Kuntz, unpublished data). The only major surgical complication was wound dehiscence, and this was significantly associated with resection of tumors in the interscapular region. Complete histologic resection was achieved in 97% cats, and local tumor recurrence was reported in only 11% cats. The 1-, 2-, and 3-year disease-free rates

were 91%, 86%, and 74%, respectively. These results suggest that extrapolation from the surgical management of canine soft tissue sarcomas and the recommendations of the Vaccine-Associated Feline Sarcoma Task Force are inadequate for the surgical treatment of feline vaccine-associated sarcomas and better rates of complete surgical resection and local tumor control can be achieved using a more aggressive surgical approach.

Surgery and radiation therapy

As a result of the high rate of local tumor recurrence following wide surgical resection, full-course radiation therapy is considered essential in the management of cats with vaccine-associated sarcoma.[194,204-206] The timing of surgery and radiation therapy is controversial. The advantages of preoperative radiation include greater antitumor effect because of a smaller population of resistant hypoxic cells as blood supply to the tumor is not disturbed, reduction in tumor size which facilitates surgical resection, and decreased risk of disseminating tumor cells during surgery.[149] The principal disadvantage of preoperative radiation therapy is the increased risk of surgical complications, such as wound dehiscence.[149] Postoperative radiation therapy may provide better tumor control, because radiation therapy is more effective against microscopic disease than gross tumor and does not delay definitive surgery.[149] However, surgery increases the size of the radiation field and increases the population of radioresistant hypoxic tumor cells by altering the local blood supply, and there is a risk of tumor cells repopulating in the interval between surgery and the start of radiation therapy.[149,194]

In two studies investigating preoperative radiation therapy, local tumor recurrence was reported in 40% to 45% of cats at a median of 398 to 584 days postoperatively.[204,206] In both studies, complete resection significantly improved the time to local recurrence with a 700 to 986 day median disease-free interval for completely excised tumors and 112 to 292 day median disease-free interval for tumors resected with incomplete margins (Figure 20-17). However, despite the prolonged interval to local tumor recurrence, complete resection following preoperative radiation therapy does not appear to improve local control rates as local tumor recurrence was reported in 42% of 59 cats with complete margins and 32% of 33 cats with incomplete margins.[206]

The outcome following postoperative radiation therapy is similar to preoperative radiation therapy. In one study, local tumor recurrence was reported in 41% of 76 cats at a median of 405 days postoperatively.[204] In another study, investigating the effects of chemotherapy in cats treated with surgery and postoperative radiation therapy, local tumor recurrence occurred in 28% of 25 cats with the median time to first recurrence not reached in cats treated with surgery
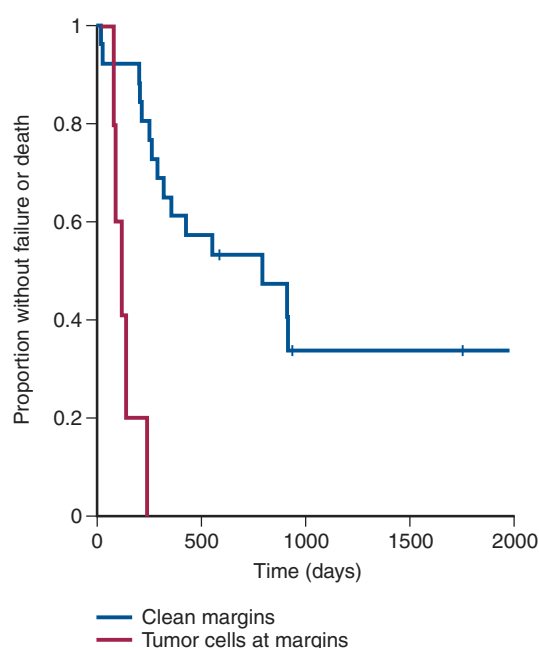
Figure 20-17

Kaplan-Meier curve for local tumor recurrence, metastasis, or death in 33 cats with vaccine-associated sarcomas treated with preoperative radiation therapy and surgical resection based on completeness of surgical margins. *(Reprinted with permission from K. Cronin, R. L. Page, G. Spodnick G, et al., "Radiation therapy and surgery for fibrosarcoma in 33 cats," Vet Radiol Ultrasound 39:51, 1998.)*

and radiation therapy alone and 661 days in cats also treated with doxorubicin.[205] Importantly, radiation therapy should start 10 to 14 days postoperatively as disease-free interval and survival time decreases as the interval between surgery and starting radiation therapy increases.[194] Local tumor recurrence does not influence survival time and, regardless of the timing of radiation therapy relative to surgery, survival data are encouraging with median survival times of 600 to 842 days and 1-, 2-, and 3-year survival rates of 86%, 44%, and 28%, respectively.[194,204,205]

Local tumor control is still disappointing with 28% to 45% of tumors recurring following multimodality treatment with surgery and radiation therapy.[194,204-206] The radiation field used in these studies typically included a minimum of 3-cm margins around the tumor or surgical scar. The majority of tumors recur within the radiation field, although tumors have been reported to recur outside the radiation field.[205] Similar to surgery alone, a more aggressive approach may be warranted to improve local tumor control, such as higher radiation doses, larger radiation fields, and more aggressive surgical resections.
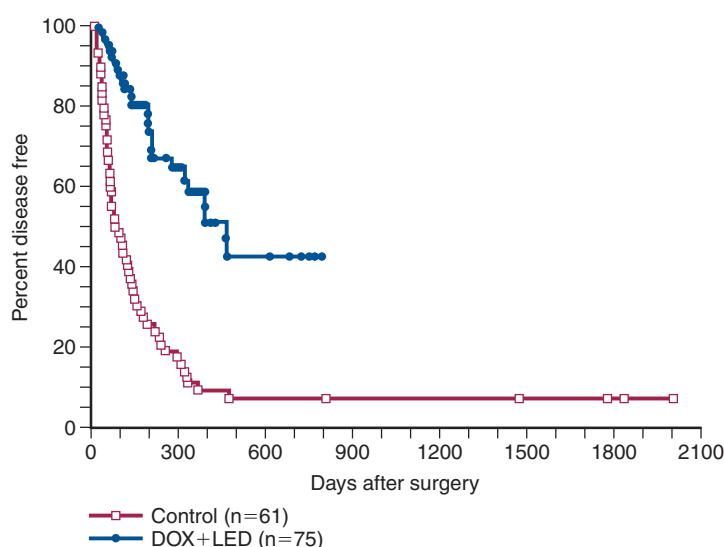
Figure 20-18

Kaplan-Meier curve for disease-free interval in 75 cats with vaccine-associated sarcomas treated with surgery and postoperative chemotherapy (liposome-encapsulated doxorubicin or doxorubicin) or surgery alone (historical control). *(Reprinted with permission from V. J. Poirier, D. H. Thamm, I. D. Kurzman, et al., "Liposome-encapsulated doxorubicin (Doxil) and doxorubicin in the treatment of vaccine-associated sarcomas in cats," J Vet Intern Med 16:726, 2002.)*

Chemotherapy

The role of chemotherapy in the management of cats with vaccine-associated sarcoma remains undefined. Metastasis has been reported in 12% to 26% of cats with vaccine-associated sarcomas, despite the aggressive histologic appearance and prevalence of high-grade lesions in these tumors, with a median time to metastasis of 265 days.[194,199,200,204,206] Vaccine-associated sarcoma cell lines have shown *in vitro* sensitivity at clinically relevant doses to doxorubicin, mitoxantrone, vincristine, and paclitaxel.[207,208] Clinically, partial and complete responses to doxorubicin, either alone or in combination with cyclophosphamide, have been reported in 39% to 50% of cats with gross tumors, but these responses are often short-lived with a median duration of 84 to 125 days.[209,210] However, median survival times are significantly prolonged in cats that respond to chemotherapy with 242 days for responders and 83 days for nonresponders.[209]

Postoperative chemotherapy has minimal impact on survival in cats treated with curative-intent surgery and radiation therapy.[204,205,210] Chemotherapy may, however, have beneficial effects on local tumor control and time to local tumor recurrence. Doxorubicin and liposome-encapsulated doxorubicin significantly prolongs the disease-free interval following surgery, with a median disease-free interval of 393 days for cats receiving chemotherapy and 93 days for those in which chemotherapy was not administered (Figure 20-18).[210]

The completeness of surgical margins may be a confounding factor in this analysis as the median disease-free interval was not reached in greater than 449 days in cats with complete surgical margins compared to 281 days following incomplete resection.[210] Carboplatin was associated with an insignificant but numerically superior median disease-free interval of greater than 986 days in cats treated with preoperative radiation therapy and surgery.[206] Other studies have shown no effect of adjunctive chemotherapy on either local tumor control or survival time.[204,205]

Immunotherapy, using recombinant viruses expressing interleukin-2 (IL-2), has shown some promise in improving local tumor control rates in cats with vaccine-associated sarcomas. Following surgical resection and iridium-based radiation therapy, the 1-year local tumor recurrence rate was 61% in cats receiving no adjunctive treatment, 39% in cats administered human IL-2 using a vaccinia virus vector, and 28% with feline IL-2 using a canary pox virus vector.[211] Other immunotherapeutic agents, such as IL-12 and acemannan, have been investigated with less favorable results.[212-214]

Prognosis

It has become clear that prognosis for cats with vaccine-associated sarcomas treated with surgical excision alone, using traditional recommendations, is poor,

especially when the first surgery is marginal and multiple surgeries are employed as the method of treatment. The median time to first recurrence was 2 months for cats treated by conservative surgery (referring veterinarian) and 9 months for those cats treated by more aggressive surgery (referral institution); the overall survival time was 19 months.[200] Another study of single modality surgical excision reported an overall disease-free interval of 10 months, which increased to longer than 16 months when excision was complete.[199]

Outcomes are improved when cats with vaccine-associated sarcomas are treated with multimodality therapy. Optimal outcome occurs when cats are treated aggressively initially and not after multiple failed resections. The combination of radiotherapy either preoperatively or postoperatively has improved the disease-free interval and overall survival time in cats with vaccine-associated sarcomas. Disease-free interval and median survival times range from 13 to 19 months and 23 months, respectively.[194,203-205] Vaccine-associated sarcomas are locally invasive; however, approximately 15% to 24% will metastasize to the lungs or other sites.[196,200,204] Continued research into the treatment of vaccine-associated sarcomas is needed as there is room for improvement in outcomes.

Prevention

The recommendations on preventing vaccine-associated sarcomas in cats are controversial. These include changing the sites of vaccine administration, decreasing the use of polyvalent vaccines, using nonadjuvanted vaccines, avoiding the use of aluminum-based adjuvants, and increasing the interval between vaccinations.[24,171,215]

The Vaccine-Associated Feline Sarcoma Task Force has recommended that no vaccine be administered in the interscapular region, rabies vaccines be administered in the distal aspect of the right pelvic limb, FeLV vaccines be administered in the distal aspect of the left pelvic limb, and all other vaccines be administered on the right shoulder.[24] The location of each injection, type of vaccine, and the manufacturer and serial number of the vaccine should be documented in the patient records. These recommendations are intended to provide epidemiologic information rather than to prevent vaccine-associated sarcomas. Vaccines should be administered into the distal, rather than mid to proximal, aspects of the limb to aid in earlier detection and increase the chance of achieving complete resection. Subcutaneous and intramuscular administration can both cause local inflammatory reactions and result in the development of vaccine-associated sarcomas.[24] Subcutaneous administration is preferred to intramuscular injection as vaccine-associated sarcomas developing from subcutaneous sites are more readily palpable and diagnosed earlier in the course of disease.

The Vaccine-Associated Feline Sarcoma Task Force has recommended that masses at vaccination sites be treated if the mass is evident ≥3 months after vaccination, >2 cm in diameter, or is increasing in size >4 weeks after vaccine administration.[181]

Traditionally, annual boosters have been recommended for most vaccines in cats. The United States Drug Authority-Animal Plant Health Inspection Service (USDA-APHIS) does not require duration of immunity studies for licensing of vaccines. However, if a duration of immunity study has not been performed, the USDA-APHIS requires that vaccine labels include a recommendation for annual revaccination.[181] The profession has questioned vaccine practices, and this has been supported by duration of immunity studies. The duration of immunity for a single commercially available inactivated, adjuvanted combination of feline panleukopenia, herpesvirus, and calicivirus is greater than 7 years with persistent antibodies against all three viruses for more than 3 years.[216,217] Local and state requirements often mandate annual rabies boosters, despite a duration of immunity of at least 3 years, because of the significant public health concern of rabies infection.[181] The Vaccine-Associated Feline Sarcoma Task Force has recommended that the administration of vaccines is a medicinal procedure and vaccination protocols should be customized for individual cats.[181] A vaccine should not be administered until the medical importance and zoonotic potential of the infectious agent, risk of exposure, and legal requirements have been considered and balanced against the risk of vaccine-associated sarcomas and other adverse effects.[181]

COMPARATIVE ASPECTS[35]

In general, soft tissue sarcomas have a similar pathologic appearance, clinical presentation, and behavior in humans and animals. However, a higher incidence is seen in young people as opposed to animals, with the exception of rhabdomyosarcoma, which is seen in young dogs. The distribution of soft tissue sarcoma in humans is similar to animals. In humans, 43% are in the extremities with two-thirds occurring in the lower limb, and 34% are intraperitoneal with 19% visceral in origin and 15% retroperitoneal. Soft tissue sarcoma of the trunk occurs in 10% of human patients, and the remaining 13% occur at other sites. Metastasis is generally hematogenous and appears to be more common in human soft tissue sarcoma than in dogs, which may partially be explained by the higher numbers of PNST (with lower metastatic rate) seen in the dog.

Most sarcomas recognized in humans are also diagnosed in animals, although the specific incidences may vary markedly. There are many more histologic subtypes recognized in humans, which are site dependent.

With the exception of benign smooth muscle tumors and subcutaneous lipomas, there is little evidence that these lesions arise from their mature (differentiated) tissue counterparts. One current theory is that switching on a set of genes that programs mesenchymal differentiation in any mesenchymal cell may give rise to any type of mesenchymal tumor. Common subtypes of soft tissue sarcoma seen in the extremities of humans are liposarcoma, MFH, synovial cell sarcoma, and fibrosarcoma. In the retroperitoneal location, liposarcoma and leiomyosarcoma are the most common histiotypes noted in humans. The most common subtype noted viscerally is the gastrointestinal stromal tumor. Previously all gastrointestinal sarcomas were classified as leiomyosarcomas or leiomyoblastomas. Currently these tumors are better classified as gastrointestinal stromal tumors because human gastrointestinal sarcomas do not express markers of myogenic differentiation. Overall, leiomyosarcoma is the most common genitourinary sarcoma. From 10% to 15% of all sarcomas occur in children, and the subtypes most commonly represented are rhabdomyosarcomas, Ewing's sarcoma, and primitive neuroectodermal tumors.

Prognostic variables in humans include clinical stage, histologic grade, necrosis, site, size, lymph node involvement, and aggressiveness of surgery or radiation. The histopathologic grading system used and shown to be predictive for metastasis in dogs is a grading system adopted from human pathology that is also predictive for survival.[24] Additionally, it appears that histologic grade is the predominant predictor of early recurrence, while tumor size plays a more important role for late recurrence. It is unclear whether age plays a prognostic role in human soft tissue sarcoma.

Surgical treatment is the mainstay of therapy for soft tissue sarcoma in the control of local disease. However, aggressive surgical resection combined with radiation therapy and chemotherapy, used before or after resection, allows effective limb-sparing treatment in 90% of patients. Amputation is reserved for patients with unresectable tumors, no evidence of metastasis, and the potential for good long-term rehabilitation. Local recurrence is greater in those patients undergoing limb-sparing therapies as compared to amputation; however, there is no difference in disease-free survival between the two groups. Since size is a prognostic factor for both local recurrence and metastasis, the recommended treatment is different. In patients with tumors less than 5 cm, complete surgical resection with 1 cm margins is usually sufficient. Larger lesions are treated with a combination of therapies, including chemotherapy and radiotherapy. Radiation therapy techniques include external beam or brachytherapy. Since half the human patients develop distant metastasis, despite local control, adjuvant chemotherapy is often used (doxorubicin alone or in combination with other chemotherapuetics). Ifosfamide

is currently the most active salvage agent for patients who have failed doxorubicin-based protocols. Meta-analysis of combination therapy for soft tissue sarcoma reports a disease-free survival of 52% and an overall survival of 57% with a median follow-up of 9.4 years. Permanent local control with the first treatment is related to long-term survival. High-risk soft tissue sarcoma patients are treated with combined chemoradiation prior to surgical resection. The chemotherapy protocol often used is doxorubicin, ifosfamide, mesna, and dacarbazine. Local and systemic toxicity included expected wound-healing complications. In human patients with metastatic disease, combination chemotherapy produces response rates of 20%, and most patients are candidates for investigational agents.

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CHAPTER 21

Cancer of the Gastrointestinal Tract

SECTION A
Oral Tumors

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INCIDENCE AND RISK FACTORS

Oral cancer accounts for 6% of canine cancer and is the fourth most common cancer overall.[1] In the cat, it accounts for 3% of all cancers.[2] Oropharyngeal cancer is 2.6 times more common in dogs than cats and male dogs have a 2.4 times greater risk of developing oropharyngeal malignancy compared to female dogs.[3,4] A male sex predisposition has also been reported for dogs with malignant melanoma and tonsillar squamous cell carcinoma (SCC).[5,6] Dog breeds with the highest risk of developing oropharyngeal cancer include the cocker spaniel, German shepherd dog, German shorthaired pointer, Weimaraner, golden retriever, Gordon setter, miniature poodle, chow chow, and boxer.[5,7,8] In one study, German shepherd dogs and boxers had a decreased risk of developing oral melanoma.[8]

In dogs, the most common malignant oral tumors are, in descending order, malignant melanoma, SCC, and fibrosarcoma.[9-20] Squamous cell carcinoma is the most common oropharyngeal cancer in cats, followed by fibrosarcoma.[2] Other malignant oral tumors in dogs include osteosarcoma, chondrosarcoma, anaplastic sarcoma, multilobular osteochondrosarcoma, intraosseous carcinoma, myxosarcoma, hemangiosarcoma, lymphoma, mast cell tumor, and transmissible venereal tumor.[9-23] Tumors or tumor-like lesions of unusual sites, types, and biologic behavior are covered at the end of this chapter (e.g., tonsillar SCC, tongue, malignancy of young dogs, viral papillomatosis, canine and feline eosinophilic granuloma complex, epulis, inductive fibroameloblastoma, and nasopharyngeal polyps). A general summary of the common oral tumors is found in Table 21-1.

PATHOLOGY AND NATURAL BEHAVIOR

The oral cavity is a common site for a wide variety of malignant and benign cancers. Although most cancers are fairly straightforward histologically, some have confusing nomenclature or extenuating circumstances that warrant discussion.

Malignant Melanoma

In comparison to other malignant oral tumors, malignant melanoma tends to occur in smaller body weight dogs. Cocker spaniels, miniature poodles, Anatolian sheepdogs, Gordon setters, chow chows, and golden retrievers are overrepresented breeds.[8] A male predisposition has been reported, but this is not a consistent finding.[6,8] The mean age at presentation is 11.4 years.[8] Malignant melanoma occurs in cats but is uncommon.[24]

Malignant melanoma can present a confusing histopathologic picture if the tumor or the biopsy section does not contain melanin, and amelanotic melanomas represent one third of all cases. A histopathologic diagnosis of undifferentiated or anaplastic sarcoma or even epithelial cancer should be looked upon with suspicion for possible underlying melanoma. Melan A is an immunohistochemical stain that may aid in the diagnosis of melanoma in dogs and can be used to differentiate melanoma from other poorly differentiated oral tumors.[8] However, while Melan A is specific for melanoma, its sensitivity drops as the tumor becomes more and more undifferentiated.

Melanoma of the oral cavity is a highly malignant tumor with frequent metastasis to regional lymph nodes and then the lungs.[6,25,26] The metastatic rate is site, size, and stage dependent and reported in up to 80% of dogs.[6,9,17,18,25-34] The World Health Organization's clinical staging system for oral tumors in dogs may have prognostic significance in dogs with oral melanoma (Table 21-2).[6,25,30,35-37] Malignant melanoma is an

TABLE 21-1   Summary of Common Oral Tumors in the Dog and Cat[10-20,27-31,36,51,55-57,73,77-79,95-103]

	Canine				Feline	
	Malignant Melanoma	Squamous Cell Carcinoma	Fibrosarcoma	Acanthomatous Epulis	Squamous Cell Carcinoma	Fibrosarcoma
Frequency	30%-40%	17%-25%	8%-25%	5%	70%-80%	13%-17%
Median age (years)	12	8-10	7-9	8	10-12	10
Sex predisposition	None-Male	None	Male	None	None	None
Animal size	Smaller	Larger	Larger	None	-	-
Site predilection	Gingiva, buccal, and labial mucosa	Rostral mandible	Maxillary gingiva and hard palate	Rostral mandible	Tongue, pharynx, and tonsils	Gingiva
Lymph node metastasis	Common (41-74%)	Rare (<40%) tonsillar SCC up to 73%	Occasional (9-28%)	None	<25%	Rare
Distant metastasis	Common (14-92%)	Rare (<36%)	Occasional (0-71%)	None	Rare	Rare (<20%)
Gross appearance	Pigmented (67%) or amelanotic (33%), ulcerated	Red, cauliflower, ulcerated	Flat, firm, ulcerated	Red, cauliflower, ulcerated	Proliferative, ulcerated	Firm
Bone involvement	Common (57%)	Common (77%)	Common (60-72%)	Common (80-100%)	Common	Common
Surgery response	Fair to good	Good	Fair to good	Excellent	Poor-high	Fair
Local recurrence	0-59%	0-50%	31-60%	0-11%		
MST	5-17 months	9-26 months	10-12 months	>28-64 months	45 day-10%	
1-year survival rate	21-35%	57-91%	21-50%	72-100%		
Radiation response	Good	Good	Poor to fair	Excellent	Poor	Poor
Response rate	83-94%	-	-	-		
Local recurrence	11-27%	31-42%	32%	8-18%		
MST	4-12 months	16-36 months	7-26 months	37 months	90 days	
1-year survival rate	36-71%	72%	76%	>85%		
Current standard of care	Surgery and/or radiation ± chemotherapy ± immunotherapy	Surgery and/or radiation	Surgery and/or radiation	Surgery	Surgery and radiation ± sensitizer	Surgery and/or radiation
Prognosis	Fair to good	Good-excellent	Good	Excellent	Poor-fair	Fair
MST	<36 months	26-36 months	18-26 months	>64 months	6-12 months	
Cause of death	Distant disease	Local or distant disease	Local disease	Rarely tumor related	Local disease	Local disease

TABLE 21-2	Clinical Staging (TNM) of Oral Tumors in Dogs and Cats[35]

Clinical staging system for oral tumors

Primary Tumor (T)

　Tis Tumor *in situ*

　T1　Tumor <2 cm in diameter at greatest dimension

　　T1a Without evidence of bone invasion

　　T1b With evidence of bone invasion

　T2　Tumor 2-4 cm in diameter at greatest dimension

　　T2a Without evidence of bone invasion

　　T2b With evidence of bone invasion

　T3　Tumor >4 cm in diameter at greatest dimension

　　T3a Without evidence of bone invasion

　　T3b With evidence of bone invasion

Regional Lymph Nodes (N)

　N0　No regional lymph node metastasis

　N1　Movable ipsilateral lymph nodes

　　N1a No evidence of lymph node metastasis

　　N1b Evidence of lymph node metastasis

　N2　Movable contralateral lymph nodes

　　N2a No evidence of lymph node metastasis

　　N2b Evidence of lymph node metastasis

　N3　Fixed lymph nodes

Distant Metastasis (M)

　M0　No distant metastasis

　M1　Distant metastasis [specify site(s)]

Stage Grouping	Tumor (T)	Nodes (N)	Metastasis (M)
I	T1	N0, N1a, N2a	M0
II	T2	N0, N1a, N2a	M0
III	T3	N0, N1a, N2a	M0
IV	Any T	N1b	M0
	Any T	N2b, N3	M0
	Any T	Any N	M1

immunogenic tumor, and molecular approaches to treatment, particularly immunomodulatory therapy, represent an active area of research.[32,33,38-46a,46b] The biology and molecular mechanisms of canine melanoma development and progression have been reviewed.[47,48]

Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) is the most common oral tumor in cats and the second most common in dogs.[1,2,17-20] In cats, the risk of developing oral SCC increases more than 3.5-fold with the use of flea collars and high intake of either canned food in general or canned tuna fish specifically.[49] Exposure to household tobacco smoke increases the risk of oral SCC twofold in cats,[49] and although this was not statistically significant, secondhand smoke exposure significantly increases expression of mutated p53 in SCC lesions compared to cats with oral SCC not exposed to environmental smoke and may be involved in tumor development and progression.[50]

Squamous cell carcinoma frequently invades bone in both cats and dogs and the degree of invasion is usually severe and extensive in the cat. Paraneoplastic hypercalcemia has been reported in two cats with oral SCC.[51] The metastatic rate in the cat is uncommon but the true incidence is unknown since so few veterinary interventions achieve local disease control that would allow long-term assessment of tumor biology. The metastatic rate for nontonsillar SCC in dogs is approximately 20%,[31] but the metastatic risk is site dependent; the rostral oral cavity having a low metastatic rate and the caudal tongue and tonsil having a high metastatic potential.

Fibrosarcoma

Oral fibrosarcoma (FSA) is the second most common oral tumor in cats and the third most common in dogs.[1,2,17-20,52] In dogs, oral FSA tends to occur in large-breed dogs, particularly the golden and Labrador retriever; at a younger age, with a median of 7.3 to 8.6 years; and with a possible male predisposition. Oral FSA will often look surprisingly benign histologically and, even with large biopsy samples, the pathologist will find it difficult to provide a diagnosis other than fibroma or low-grade FSA. This syndrome, which is common on the hard palate (Figure 21-1) and maxillary arcade between the canine and carnassial teeth of large-breed dogs, has been termed "histologically low-grade but biologically high-grade" FSA (or "high-low" FSA).[52] Even with a biopsy result suggesting fibroma or low-grade FSA, the treatment should be aggressive, especially if the cancer is rapidly growing, recurrent, or invading bone. FSA is very locally invasive but metastasizes to the lungs and regional lymph nodes in less than 30% of dogs.[9,17-20,31]

Epulides

Fibromatous and ossifying epulides

Epulides are relatively common in dogs, but they are rare in cats. Multiple epulides have been described in cats with 50% of cases occurring in cats younger than 3 years.[53] Epulides are benign gingival proliferations arising from the periodontal ligament and appear similar to gingival hyperplasia (Figure 21-2). Four types of epulides have been described in the dog: fibromatous, ossifying, acanthomatous, and giant cell.[54-56] The mean age at presentation for dogs with fibromatous and ossifying epulides is 8 to 9 years, and a male predisposition was reported in one study.[55,56] Fibromatous and
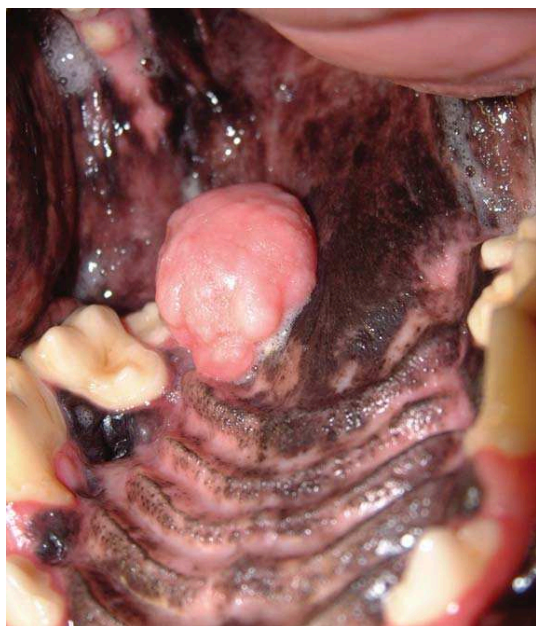
Figure 21-1

A histologically low-grade but biologically high-grade fibrosarcoma in the palate of a golden retriever. The tumor appears circumscribed and benign, but aggressive surgical resection and possibly postoperative radiation therapy are required for local tumor control.

Figure 21-2

Typical appearance of a fibromatous epulis. The mass is firmly adhered to the underlying bone but does not invade bone. Conservative resection and cryosurgery were curative in this dog.

ossifying epulides are slow-growing and firm masses that are usually covered by intact epithelium. They have a predilection for the maxillary premolar teeth.[56] Ossifying epulides usually have a broad base of attachment and are less pedunculated than fibromatous epulides.[56] These two epulides are histologically differentiated by the presence or absence of bone.[54]

Acanthomatous epulides

Acanthomatous epulis has an aggressive local behavior and frequently invades bone of the underlying mandible or maxilla. Shetland and Old English sheepdogs are predisposed.[56,57] The mean age at presentation is 7 to 10 years, and a sex predisposition is unlikely with three studies reporting conflicting results.[55-58] The rostral mandible is the most common site.[57] They do not metastasize. *Acanthomatous epulis* is the preferred term, but some pathologists will refer to these tumors by their previous terminology of adamantinoma.[54]

HISTORY AND SIGNS

Most cats and dogs with oral cancer present with a mass in the mouth noticed by the owner. Owners rarely see cancer in the caudal pharynx, and animals present for signs of increased salivation, exophthalmos or facial swelling, epistaxis, weight loss, halitosis, sanguinous oral discharge, dysphagia or pain on opening the mouth, or cervical lymphadenopathy (especially SCC of the tonsil).[17-20,59] Loose teeth, especially in an animal with generally good dentition, should alert the clinician to possible underlying neoplastic bone lysis (Figure 21-3).[60] Although paraneoplastic syndromes associated with oral tumors are rare, hypercalcemia has been reported in two cats with oral SCC[51] and hyperglycemia in a cat with a gingival vascular hamartoma.[61]

DIAGNOSTIC TECHNIQUES AND WORKUP

The diagnostic evaluation for oral cancers is critical due to the wide ranges of cancer behavior and therapeutic options available. If the cancer is suspected of being malignant, thoracic radiographs can be performed prior to biopsy. Most animals will require a short general anesthesia for careful palpation, regional radiographs, and a biopsy.

Cancers that are adherent to bone, other than fibromatous or ossifying epulides, should have regional radiographs taken under general anesthesia. Regional radiographs include open mouth, intraoral, oblique lateral, and ventrodorsal or dorsoventral projections.[62] Bone lysis is not radiographically evident until 40% or more of the cortex is destroyed (Figure 21-4). However, apparently normal radiographs do not exclude bone invasion. This evaluation will assist in determining clinical staging information and the extent of resection when surgery is indicated. Computed tomography (CT)

Figure 21-3

An intraoral radiograph of the rostral mandible of a dog with an acanthomatous epulis. Note the bone lysis and loosening of the incisor teeth (*arrows*).

Figure 21-4

An intraoral radiograph of the rostral mandible of a cat with a squamous cell carcinoma. Note the extensive bone lysis, which is common in cats with this type of tumor.

(or magnetic resonance imaging in specific cases) can be a valuable staging tool, especially for evaluation of bone invasion and possible tumor extension into the nasal cavity or in the caudal pharynx and orbit (Figure 21-5). The use of CT may preclude the necessity for radiographs of the primary lesion.

Regional lymph nodes should be carefully palpated for enlargement or asymmetry; however, lymph node size is not an accurate predictor of metastasis. In 100 dogs with oral melanoma, 40% of dogs with normal sized lymph nodes had metastasis and 49% with enlarged lymph nodes did not have metastasis.[26] Regional lymph nodes include the mandibular, parotid, and medial retropharyngeal lymph nodes; however, the parotid and medial retropharyngeal lymph nodes are not normally palpable.[63] Furthermore, only 55% of 31 cats and dogs with metastasis to the regional lymph nodes had metastasis to the mandibular lymph node.[64] Lymphoscintigraphy or contrast-enhanced ultrasonography can be used to detect sentinel lymph nodes and guide lymph node aspirates.[65] Lymph node aspirates should be performed in all animals with oral cancers, regardless of the size or degree of fixation of the lymph nodes.[26,64] En bloc resection of

regional lymph nodes has been described and, although the therapeutic benefit of this approach is unknown, it may provide valuable staging information.[63,64] Based on these diagnostic steps, oral tumors are then clinically staged according to the World Health Organization's staging scheme (see Table 21-2).[35]

The final step, under the same anesthesia, is a large incisional biopsy. Cytologic touch or aspiration preparations of oral tumors are usually not rewarding because of the necrosis and inflammation that commonly accompany these cancers. Dogs with exophytic or ulcerated masses will generally tolerate a deep wedge or core punch biopsy without general anesthesia. Biopsy is recommended to differentiate benign from malignant disease, for owners basing their treatment options on prognosis, and when other treatment modalities, such as radiation therapy, are being considered. Oral cancers are commonly infected, inflamed, or necrotic, and it is important to obtain a large specimen. Electrocautery may distort the specimen and should only be used for hemostasis after blade incision or punch biopsy. Large samples of healthy tissue at the edge and center of the lesion will increase the diagnostic yield, but care must
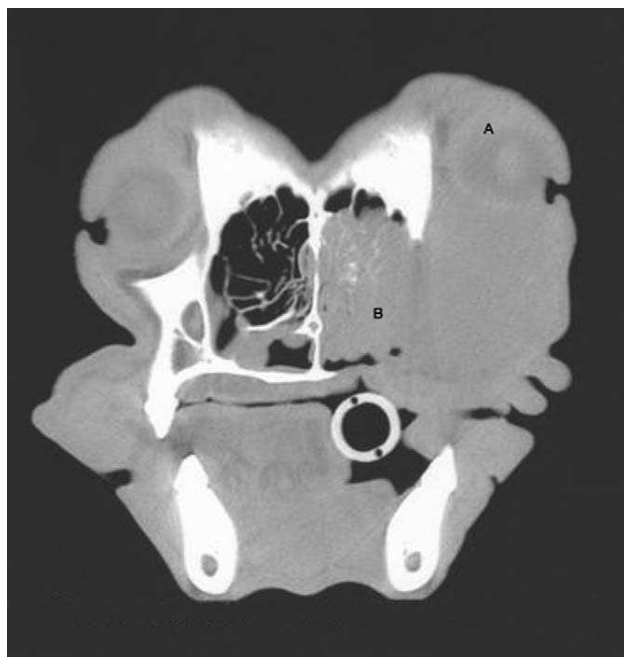
Figure 21-5

A CT image of a dog with a maxillary fibrosarcoma. Advanced imaging allows better planning of surgery and radiation therapy as the extent of bone involvement and extension into the nasal cavity is often much greater than can be appreciated grossly. The tumor is displacing the eye (*A*) dorsally and laterally and is extending into the nasal cavity (*B*).

be taken not to contaminate normal tissue, which cannot be removed with surgery or included in the radiation field. Biopsies should always be performed from within the oral cavity and not through the lip to avoid seeding tumor cells in normal skin and compromising curative-intent surgical resection. For small lesions (e.g., epulides, papillomas, or small labial mucosal melanoma), curative-intent resection (excisional biopsy) may be undertaken at the time of initial evaluation. For more extensive disease, waiting for biopsy results to accurately plan treatment is strongly encouraged.

THERAPY

Surgery

Surgery and radiation therapy are the most common treatments used for the local control of oral tumors. Surgical resection is the most economical, fastest, and most curative treatment available for most localized tumors. The type of oral surgery depends on tumor type and location. Except for fibromatous and ossifying epulides, most oral tumors have some underlying bone involvement and surgical resection should include bony margins to increase the rate of local tumor control. Mandibulectomy (segmental or hemi), segmental maxillectomy, and orbitectomy are generally well tolerated by cats and dogs. These procedures are indicated for most oral tumors, particularly lesions with extensive bone invasion, poor sensitivity to radiation therapy, or too large for cryosurgery (Tables 21-3 and 21-4).[10-20,66-68] Margins of at least 2 cm are usually necessary for malignant cancers such as SCC, malignant melanoma, and fibrosarcoma in the dog. If possible, SCC in the cat should be treated with surgical margins greater than 2 cm because of high local recurrence rates. Bony reconstruction following bony resection has been described but is rarely necessary.[12,70-72] Rostral and segmental resections (e.g., mandibulectomy and maxillectomy) may be sufficient for benign lesions and rostral SCC in dogs. Larger resections, including hemimandibulectomy, hemimaxillectomy, orbitectomy, and radical maxillectomy, may be necessary for more aggressive tumors, especially fibrosarcoma, and malignant tumors with a more caudal location.[10-20,66-68] Although these large resections carry some morbidity, owner satisfaction with the cosmetic and functional outcomes are in excess of 85%.[10-20,67,69,73] Cosmetic appearance is usually very good following most mandibulectomy and maxillectomy procedures (Figure 21-6) but can be challenging with aggressive bilateral rostral mandibulectomies and radical maxillectomies.[10-20,66-68] Blood loss and hypotension are the most common intraoperative complications, particularly during caudal or aggressive maxillectomy procedures.[18,67] Presurgical blood typing and cross-matching to ensure adequate availability of blood products during surgery is recommended. Postoperative complications include incisional dehiscence, epistaxis, increased salivation, mandibular drift and malocclusion, and difficulty prehending food, particularly after bilateral rostral mandibulectomy caudal to the second premolar teeth.[12-20,51,67] Enteral feeding tubes are not usually required following oral surgery in dogs but are often recommended for cats treated with any type of mandibulectomy as eating can be difficult for 1 to 2 months following surgery.[51,73]

Local disease control is the goal of treatment for most animals with oral tumors. Regional lymph node resection has been described in cats and dogs and, although it adds to clinical staging information, its effectiveness in controlling local and metastatic disease is unknown.[63,64] If postoperative adjuvant therapy (e.g., radiation therapy or chemotherapy) is being contemplated, regional nodes having confirmed or suspicious metastasis should be extirpated to achieve minimal residual disease.

TABLE 21-3   Various Mandibulectomies

Mandibulectomy Procedure	Indications	Comments	
Unilateral rostral	Lesions confined to rostral hemimandible; not crossing midline	Most common tumor types are squamous cell carcinoma and adamantinoma that do not require removal of entire affected bone; tongue may lag to resected side	
Bilateral rostral	Bilateral rostral lesions crossing the symphysis	Tongue will be "too long" and some cheilitis of chin skin will occur; has been performed as far back as PM4 but preferably at PM1	
Vertical ramus	Low-grade bony or cartilaginous lesions confined to vertical ramus	These tumors are variously called chondroma rodens or multilobular osteosarcoma; temperomandibular joint may be removed; cosmetics and function are excellent	
Complete unilateral	High-grade tumors with extensive involvement of horizontal ramus or invasion into medullary canal of ramus	Usually reserved for aggressive tumors; function and cosmetics are good	
Segmental	Low-grade midhorizontal ramus cancer, preferably not into medullary cavity	Poor choice for highly malignant cancer in medullary cavity, since growth along mandibular artery, vein, and nerve is common	

TABLE 21-4   Various Maxillectomies

Maxillectomy Procedure	Indications	Comments	
Unilateral rostral	Lesions confined to hard palate on one side	One-layer closure	
Bilateral rostral	Bilateral lesions of rostral hard palate	Needs viable buccal mucosa on both sides for flap closure	
Lateral	Laterally placed midmaxillary lesions	Single-layer closure if small defect, two-layer if large	
Bilateral	Bilateral palatine lesions	High rate of closure dehiscence because lip flap rarely reaches from side to side; may result in permanent oronasal fistula	
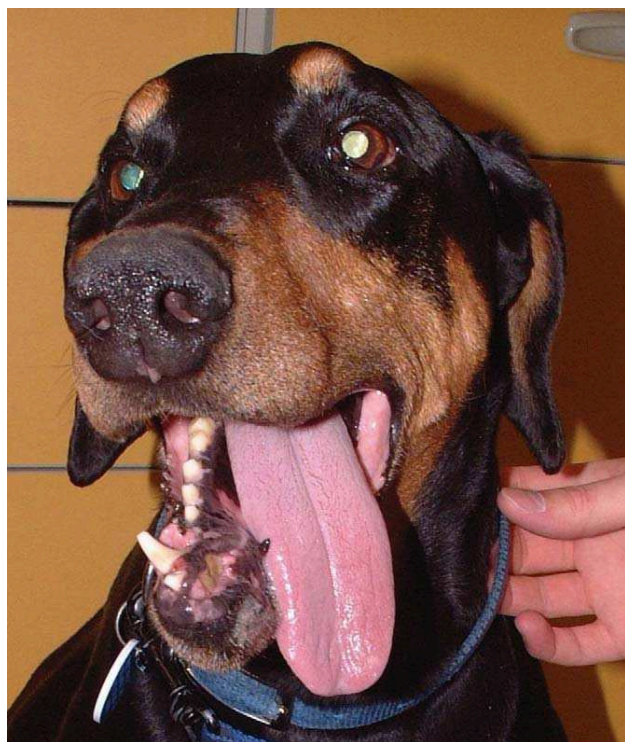
Figure 21-6

The typical appearance of a dog 6 months postoperatively after subtotal unilateral mandibulectomy for an osteosarcoma. The tongue will often hang out and the remaining hemimandible will drift toward the resected side.

Cryosurgery (Chapter 15, Section A) may be indicated for lesions less than 2 cm in diameter that are fixed or minimally invasive into bone. Larger lesions should generally be surgically resected. More extensive lesions in bone will often result in a fracture (mandible) or oronasal fistula (maxilla) if aggressively frozen. Oral cancer involving only soft tissues without bone should be surgically excised and not frozen.

Radiation Therapy

Radiation therapy can be effective for locoregional control of oral tumors. Radiation therapy can be used as primary treatment, with either palliative or curative intent, or as an adjunct for incompletely resected tumors or tumors with an aggressive local behavior, such as oral fibrosarcoma. Malignant melanoma, canine oral SCC, and some benign tumors such as the epulides, are known to be radiation responsive, and radiation therapy should be considered in the primary treatment of these tumors.[30-32,74] For canine oral SCC, dental tumors, and fibrosarcoma, daily and alternate day protocols have been described consisting of 2.7 Gy to

4.2 Gy per fraction with a total dose ranging from 48 Gy to 57 Gy.[31,75] Tumor control is better for smaller benign and malignant lesions (T1 and T2 tumors) treated with radiation alone.[30,31,74] Local tumor control and survival time should be improved by combining radiation therapy with radiation sensitizers, surgery, or chemotherapy, especially for tumors considered radiation resistant, such as canine oral fibrosarcoma and feline oral SCC.[51,76-80] Radiation sensitizers, such as etanidazole and gemcitabine, have been used in cats with oral SCC and platinum drugs have been used as radiation sensitizers in dogs with oral melanoma.[27,36,77,80]

Oral melanoma is generally responsive to coarse fractionation protocols. Four different hypofractionated radiation protocols have been described: (1) three weekly 8 to 10 Gy fractions for a total dose of 24 to 30 Gy,[29,36] (2) four weekly fractions of 9 Gy for a total dose of 36 Gy,[30,36] (3) six weekly 6 Gy fractions for a total dose of 36 Gy,[27] and (4) eight weekly 6 Gy fractions for a total dose of 48 Gy.[81] In humans, the effect of total radiation dose is controversial, but fraction dose does have an impact on response rates. Response rates are significantly better with fractions greater than 8 Gy compared to less than 4 Gy.[82] However, in one study comparing two hypofractionated protocols of 9 to 10 Gy per fraction to a fully fractionated protocol of 2 to 4 Gy per fraction, there were no significant differences in either local recurrence rates or survival time.[36] Coarse fractionation of oral melanoma has also been described in five cats with limited success, including one complete response and two partial responses.[24]

Acute effects are common but self-limiting. Depending on the tissues irradiated, these include alopecia and moist desquamation, oral mucositis, dysphagia, and ocular changes, such as blepharitis, conjunctivitis, keratitis, and uveitis.[31,74,83-85] The acute effects of coarse fractionation are less than experienced with full-course protocols and usually resolve rapidly.[30] Late complications are rare, occurring in less than 5% of cases, but can include permanent alopecia, skin fibrosis, bone necrosis and oronasal fistula formation, development of a second malignancy within the radiation field, keratoconjunctivitis sicca, cataract formation, and ocular atrophy.[31,58,74,86] Orthovoltage radiation is associated with a higher incidence of second malignancies and bone necrosis than megavoltage irradiation.[31,58,74]

Hyperthermia offers no advantage over cryosurgery or surgery if it is used alone. Bone penetration is less reproducible with heat versus cold treatment. Hyperthermia at moderate temperatures (42 to 43°C) may, however, be used as an effective adjunct to irradiation.[28,87,88]

Chemotherapy

The major problem with most oral tumors is control of local disease. However, chemotherapy is indicated for

TABLE 21-5	Postoperative Outcome Following Mandibulectomy for Different Tumor Types[10-20,55-57,109-111]				
Tumor Type	Number	Local Recurrence	Median Survival Time	1-Year Survival Rate
Malignant melanoma	81	0-40%	7-17 months	21%
Squamous cell carcinoma	74	0-23%	9-26 months	80-91%
Fibrosarcoma	58	31-60%	11-12 months	23-50%
Osteosarcoma	144	15-44%	6-18 months	35-71%
Acanthomatous epulis	116	0-3%	> 28-64 months	98-100%

some tumors associated with high metastatic potential, especially oral melanoma in dogs and tonsillar SCC in cats and dogs. Although expression of COX-2 is reported to be significantly increased in feline oral SCC,[89] nonsteroidal anti-inflammatory drugs such as piroxicam have not been effective in the management of this disease.[90] Piroxicam may have some effect against oral SCC in dogs,[91] and response rates were improved when piroxicam was combined with either cisplatin or carboplatin; however, renal toxicity generally precludes the use of cisplatin at standard doses with piroxicam.[92,93] A liposome-encapsulated form of an investigational platinum agent was not effective in cats with oral SCC,[94] but mitoxantrone, in combination with radiation therapy, has shown some potential in a small number of cats.[78,79]

In general, oral melanoma is chemoresistant in both dogs and cats. The platinum drugs have shown the most, albeit marginal, promise in treating dogs with oral melanoma, and include intralesional cisplatin[95] and systemic carboplatin.[96] Measurable responses to melphalan have also been reported in rare cases.[97] Immunomodulatory approaches, using transgenes, antitumor vaccines, immunotherapy agents, or biologic response modifiers, are under intensive scrutiny and currently hold the most promise for adjunctive treatment of oral melanoma.

Malignant melanoma is generally an immunogenic tumor. The use of immunotherapy agents and biologic response modifiers is an emerging and exciting approach for the adjunctive management of dogs with oral melanoma. Biologic response modifiers, such as *Corynebacterium parvum* and liposome-muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE), have shown encouraging results compared to control groups in controlled clinical studies.[32,33] Others, however, have failed to improve survival times in dogs with malignant melanoma using immunomodulators such as bacillus Calmette-Guérin (BCG) and levamisole. Gene-engineered immunotherapy is an active area of research, and current approaches have resulted in significant improvements in local control rates and survival times. These approaches include systemic administration of interleukin-2 (IL-2) and tumor necrosis factor;[38] intratumoral injection of xenogeneic histoincompatible human Vero cells genetically modified to produce human recombinant IL-2, a potent proinflammatory cytokine;[43] autologous vaccines from irradiated tumor cells transfected with human recombinant (hr) granulocyte-macrophage colony-stimulating factor (GM-CSF);[42] direct injection of DNA into tumors to induce expression of staphylococcal enterotoxin B (a superantigen) in combination with either GM-CSF or IL-2;[39-41] DNA vaccination with either murine or human tyrosinase; and gene-engineered autologous tumor vaccines.[44-46a,46b] Preliminary results suggest immunotherapeutic approaches, in combination with either surgery or radiation therapy, are promising in the management of oral melanoma, but large-scale clinical trials are lacking. A review of these and other immunologic approaches to treating cancer are presented in Chapter 13.

PROGNOSIS

Clinical series of more than 500 dogs with various oral malignancies treated with either mandibulectomy or maxillectomy have been described.[10-20,66-68] The majority of cases were treated with surgery alone. Unfortunately, the methods of reporting and end results vary with each paper, but an attempt to combine cases by tumor type and outcome is shown in Tables 21-5 and 21-6. Overall, the lowest rates of local tumor recurrence and best survival times are reported in dogs with acanthomatous epulis and SCC, while FSA and malignant melanoma are associated with the poorest results.[10-20] Most of these reports suggest that histologically complete resection and a rostral location are favorable prognostically. In two studies of 142 dogs treated with either mandibulectomy or maxillectomy, tumor-related deaths were 10 to

TABLE 21-6	Postoperative Outcome Following Maxillectomy for Different Tumor Types[10-20,55-57,109-111]			
Tumor Type	**Number**	**Local Recurrence**	**Median Survival Time**	**1-Year Survival Rate**
Malignant melanoma	37	21-48%	5-10 months	27%
Squamous cell carcinoma	13	29-50%	19 months	57%
Fibrosarcoma	33	33-57%	10-12 months	21-50%
Osteosarcoma	50	27-100%	4-10 months	17-27%
Acanthomatous epulis	30	0-11%	> 26-30 months	72-100%

21 times more likely with malignant tumors, up to five times more likely with tumors located caudal to the canine teeth, and two to four times more likely following incomplete resection.[19,20] Rostral locations are usually detected at an earlier stage and are more likely to be resected with complete surgical margins. Local tumor recurrence is more frequent following incomplete resection, with 15% to 22% and 62% to 65% of tumors recurring following complete and incomplete excision, respectively.[19,20] Additionally, recurrent disease negatively impacts survival time as subsequent treatment is more difficult and responses are less common.[25,34] FSA continues to have an unacceptable local recurrence rate and more aggressive resections or the addition of standard or novel adjuvant therapies (e.g., postoperative radiation, chemotherapy, immunotherapy).[75] On the other hand, melanoma is controlled locally in 75% of cases, but metastatic disease requires more effective adjuvant therapies.

For dogs treated with megavoltage radiation, tumor size is the most important factor in local tumor control. Local recurrence is reported in up to 30% of cases and, compared to T1 tumors, recurrence is three times more likely with T2 tumors and up to eight times more likely with T3 tumors.[31,74] Tumor size is also associated with survival durations in dogs with malignant oral tumors, with 3-year progression-free survival rates of 55%, 32%, and 20% for T1, T2, and T3 tumors, respectively.[31]

Malignant Melanoma

The prognosis for dogs with oral melanoma is guarded. Metastatic disease, particularly to the lungs, is the most common cause of death with metastasis to the lungs reported in 14% to 67% of dogs.[6,9,17,18,25-34] Surgery and radiation therapy can provide good control of local disease, but strategies to manage the high metastatic potential of malignant melanoma, such as chemotherapy and immunotherapy, require further investigation.

Surgery is the most common treatment for management of the local tumor. The local tumor recurrence rates varies from 22% following mandibulectomy to 48% after maxillectomy.[6,17,18] The median survival time for dogs with malignant melanoma treated with surgery alone varies from 150 to 318 days with 1-year survival rates less than 35%.[6,8,17-20,25,33,34,57] When combined with chemotherapy, however, the median survival time may be modestly improved.[6,25] Regardless, tumor control and survival time are significantly better when surgery is included in the treatment plan.[37] In comparison, the median survival time for untreated dogs with oral melanoma is 65 days.[34] Variables that are known to have prognostic significance in dogs treated with surgery alone or in combination with other modalities include tumor size, clinical stage, and ability of the first treatment to achieve local control.[6,25,27,28,30-34] Dogs with tumors smaller than 2 cm diameter have a median survival time of 511 days compared to 164 days for dogs with tumors greater than 2 cm diameter or lymph node metastasis.[32] Median survival times are significantly shorter for dogs with recurrent oral malignant melanomas compared to dogs with previously untreated oral melanomas.[25,34] In some studies, tumor location also has prognostic importance with rostral mandibular sites having a better prognosis than other sites.[37,95] Age, breed, sex, degree of pigmentation, microscopic appearance, and DNA ploidy are not prognostic.

Oral melanoma is responsive to hypofractionated radiation therapy protocols. Response rates are excellent with 83% to 100% of tumors responding and a complete response observed in up to 70% of melanomas.[27,29-31,81] Local recurrence is reported in 15% to 26% of dogs achieving a complete response with a median time to local recurrence of 139 days.[27,29-31] Progressive local disease was observed in all dogs that did not achieve a complete response in one study.[29] The most common cause of death is metastasis and this is reported in 58% of dogs with a median time of metastasis of 311 days.[27] The median survival time for dogs treated with radiation therapy is 211 to 363 days with a 1-year survival rate of 36% to 48% and a 2-year survival rate of 21%.[27,29-31,36] Local tumor control and survival

time are significantly improved with rostral tumor location, smaller tumor volume, no radiographic evidence of bone lysis, and postoperative irradiation of microscopic disease.[30,31,36] When considering the risk factors of a nonrostral location, bone lysis, and macroscopic disease in one series of 140 dogs, the median survival time was 21 months and significantly better if none of these risk factors were present compared to a median survival time of 11 months with one risk factor, 5 months with two risk factors, and 3 months with all three risk factors.[36] Tumor size is important with a median progression-free survival for dogs with T1 oral melanomas of 19 months compared to less than 7 months for T2 and T3 tumors.[31] Hypofractionated radiation therapy has also been described in five cats with oral melanoma resulting in a 60% response rate and median survival time of 146 days (range, 66 to 224 days).[24]

Effective chemotherapy or immunotherapy protocols are in demand for the adjunctive management of dogs with oral melanoma because of the high metastatic risk. Unfortunately, most oral melanoma in dogs is relatively resistant to standard chemotherapy. Chemotherapy can be administered either intralesionally or systemically. Intralesional implants that release cisplatin significantly improve survival time compared to control dogs, with a median survival time of 51 weeks compared to 10 weeks, but implant-related complications were common.[95] The platinum drugs are preferred for both radiation sensitization[27,36] and systemic chemotherapy.[25,96] Both melphalan and carboplatin result in similar outcomes with an overall response rate less than 30% and a median duration of response of approximately 4 months.[96,97] Cisplatin and piroxicam have been investigated, but this treatment regimen, despite a response rate of 18%, should be used with caution because of a high rate of renal toxicosis.[92] The use of immunomodulation for melanoma is showing some encouraging results, but most results involve small numbers of dogs in phase I trials. In one study of 98 dogs with oral melanoma, the 2-year survival rate was 80% in dogs with clinical stage I disease treated with surgery and the biologic response modifier L-MTP-PE compared to 25% for dogs treated with surgery alone. However, L-MTP-PE did not influence survival times in dogs with stage II and III disease.[33] Other biologic response modifiers, such as BCG and levamisole, have failed to improve survival times for dogs with malignant melanoma. Therapy involving the delivery of immunomodulating transgenes shows the most promise for the adjunctive management of dogs with oral melanoma. In a controlled study of intratumoral injection of xenogeneic human Vero cells producing hrIL-2 following surgery and radiation therapy, 92% of 12 control dogs had either tumor recurrence or metastasis by 12 months with a median survival time of 2.4 months compared to 50% remission rate in dogs treated with hrIL-2 and a median

survival time of 9 months.[43] A 46% response rate was observed in 26 dogs treated with intratumoral injection of DNA with either hrGM-CSF or hrIL-2.[39-41] The use of DNA vaccinations with either murine or human tyrosinase in dogs with advanced stages of oral melanoma (clinical stage II to IV) results in median survival times of 224 to 389 days.[44-46a] In one study of nine dogs treated with DNA vaccination and human tyrosinase, complete response was observed in one dog with lung metastasis, two dogs with stage IV disease and bulky metastasis lived for greater than 400 days, and two dogs with stage II or III disease died of other causes approximately 500 days after treatment with no evidence of tumor at necropsy.[45] The median survival time is significantly improved to 589 days when the primary oral site and regional lymph nodes are controlled with surgery or radiation therapy.[44] In another clinical trial, a xenogeneic melanoma-antigen (gp100) enhanced allogeneic tumor cell vaccine resulted in an overall complete and partial response rate of 17% and a tumor control rate of 35%.[46b] Several studies are currently ongoing to improve the efficacy of melanoma tumor vaccines.

The location of malignant melanoma may also have some prognostic significance. Melanomas of the lip and tongue have a lower metastatic rate and survival is more dependent on local control of the tumor. In one series of 60 dogs with oral melanomas at various sites treated with combinations of surgery, radiation therapy, chemotherapy and immunotherapy, the median survival time for dogs with lip melanomas was 580 days and greater than 551 days for dogs with tongue melanomas.[6] In comparison, the median survival time was 319 days for maxillary melanomas and 330 days for melanomas of the hard palate.[6]

Squamous Cell Carcinoma

Canine oral squamous cell carcinoma

The prognosis for dogs with oral SCC is good, particularly for rostral tumor locations. Local tumor control is usually the most important challenge, although metastasis to the regional lymph nodes is reported in up to 10% of dogs and to the lungs in 3% to 36% of dogs.[31] In contrast, SCC of the tonsils and base of the tongue are highly metastatic, with metastasis reported in up to 73% dogs, and local or regional recurrence is common.[98-100] Surgery and radiation therapy can both be used for locoregional control of oral SCC in dogs. Photodynamic therapy has also been reported with good results in 11 dogs with oral SCC.[101]

Surgery is the most common treatment for management of nontonsillar SCC.[10] Following mandibulectomy, the local recurrence rate is 10% and the median survival time varies from 19 to 26 months with a 91% 1-year survival time.[17] In comparison, the local recurrence rate is 29% after maxillectomy with a median

survival time of 10 to 19 months and a 1-year survival rate of 57%.[18] Full-course radiation therapy, either alone or as an adjunct following incomplete surgical resection, is also a successful treatment modality for the management of oral SCC in dogs.[31,102,103] The local tumor recurrence rate is 31%. The median survival time for radiation therapy alone is 15 to 16 months and increases to 34 months when combined with surgery.[102,103] In one series of 39 dogs with oral SCC, the overall median progression-free survival time was 36 months with 1- and 3-year progression-free survival rates of 72% and 55%, respectively.[31] Local tumor control was better with smaller lesions as the median progression-free survival time for T1 tumors (<2 cm diameter) was greater than 68 months compared to 28 months for T2 tumors (2 to 4 cm diameter) and 8 months for dogs with T3 tumors (>4 cm diameter).[31] Other favorable prognostic factors include rostral tumor location, maxillary SCC, and young age.[102] Rostral tumors (median survival time of 28 months compared to 2 to 10 months for caudal to extensive tumors), nonrecurrent tumors (median survival time 29 months compared to 7 months for recurrent SCC), portal size less than 100 cm^2/m^2 (median survival time 24 months compared to 7 months), and age less than 6 years (median survival time of 39 months compared to 10 months) are good prognostic indices for dogs treated with orthovoltage radiation therapy.[102] Age is also prognostic for dogs treated with megavoltage radiation as the median survival time of 315 days for dogs older than 9 years is significantly shorter than the 1080 days reported for dogs less than 9 years.[103]

Chemotherapy may be indicated for dogs with metastatic disease, bulky disease, and when owners decline surgery and radiation therapy. However, as the metastatic potential of oral SCC in dogs is relatively low, the role of chemotherapy in minimizing the risk of metastatic disease after local surgery or radiation is unknown. In one series of 17 dogs with measurable disease treated with piroxicam alone, the response rate was 17%, with one complete response and two partial responses.[91] The median progression-free interval for dogs responding to piroxicam was 180 days and significantly longer than the 102 days for dogs with stable disease.[91] The outcome is better when piroxicam is combined with either cisplatin or carboplatin. In a series of nine dogs treated with piroxicam and cisplatin, the overall median survival time was 237 days with the 56% of dogs responding to this chemotherapy protocol having a significantly better outcome than nonresponders with a median survival time of 272 days compared to 116 days.[92] However, renal toxicity was reported in 41% of dogs in this study, and such toxicities limit the clinical utility of this protocol. In another small series of seven dogs with T3 oral SCC treated with piroxicam and carboplatin, a complete response was observed in 57% of dogs, and this response was sustained in all dogs at the median follow-up time of 335 days.[93]

Feline oral squamous cell carcinoma

The prognosis for cats with oral SCC is poor.[13,59,104,105] There is no known consistently effective treatment. Local control is the most challenging problem. In one series of 52 cats, the 1-year survival rate was less than 10% with median survival times of 3 months or less for surgery alone, surgery and radiation therapy, radiation therapy and low-dose chemotherapy, or radiation therapy and hyperthermia.[59] However, 42% of these cats had SCC involving the tongue, pharynx, or tonsils. The oncologic outcome may be better for cats with mandibular SCC. The median survival time for seven cats treated with a combination of mandibulectomy and radiation therapy was 14 months with a 1-year survival rate of 57%.[51] Local recurrence was the cause of failure in 86% of these cats between 3 to 36 months after therapy. In another series of 22 cats treated with mandibulectomy alone, the median disease-free interval was 340 days.[73] Tumor location and extent of resection had prognostic importance with a median survival time of 911 days for rostral tumors, 217 days following hemimandibulectomy, and 192 days when greater than 50% of the mandible was resected.[73] The use of esophagostomy or gastrostomy tubes is important to provide supplemental nutrition in these cats postoperatively.

Radiation therapy alone is generally ineffective in the management of cats with oral SCC. However, the combination of radiation therapy with radiation sensitizers or chemotherapy improves response rates and survival times. Intratumoral etanidazole, a hypoxic cell sensitizer, resulted in a 100% partial response rate in nine cats completing the radiation therapy course with a median decrease in tumor size of 70% and a median survival time of 116 days.[77] Gemcitabine was used at low doses as a radiation sensitizer in eight cats with oral SCC with an overall response rate of 75%, including two cats with complete responses, for a median duration of 43 days and a median survival time of 112 days.[80] The combination of radiation therapy with mitoxantrone holds some promise as, in two series of 18 cats, a complete response was observed in 73% with a median duration of response of 138 to 170 days and a median survival time of 184 days.[78,79] Palliative radiation protocols, consisting of 8 Gy fractions on days 0, 7, and 21, are not recommended because of poor disease control and radiation-induced adverse effects.[106]

Chemotherapy appears ineffective in the management of cats with oral SCC. No responses were observed in 18 cats treated with a liposome-encapsulated platinum agent or 13 cats treated with piroxicam.[90,94]

Fibrosarcoma

The prognosis for dogs with oral fibrosarcoma is guarded. These are locally aggressive tumors and local control is more problematic than metastasis.

Metastasis is reported to the regional lymph nodes in 19% to 22% of dogs and to the lungs in up to 27% of dogs.[17,18,31] Multimodality treatment of the local disease appears to afford the best survival rates, with combinations of surgery and radiation therapy or radiation therapy and hyperthermia.[28,75]

Surgery is the most common treatment for oral fibrosarcoma. The median disease-free interval for five cats treated with mandibulectomy was 859 days.[73] Following mandibulectomy, local recurrence is reported in 59% of dogs with a median survival time of 11 months and a 1-year survival rate of 50%.[17] The outcome is similar following maxillectomy, with local recurrence in 40% of dogs and a median survival time of 12 months.[18] One-year survival rates rarely exceed 50% with surgery alone.[10-20] The combination of surgery and radiation therapy provides the best opportunity to control local disease.

Oral fibrosarcomas are considered radiation resistant.[76,107,108] The mean survival time of 17 dogs treated with radiation therapy alone was only 7 months.[76] Radiation therapy combined with regional hyperthermia improved local control rates to 50% at 1 year in a series of 10 cases.[28] When radiation therapy is used as an adjunct to surgical resection, local tumor recurrence was reported in 32% of dogs overall and the median survival time increased to 18 to 26 months with a 1-year progression-free survival rate of 76%.[31,75] A smaller tumor size improves the outcome following radiation therapy, with the median progression-free survival time of 45 months for dogs with T1 tumors compared to 31 months and 7 months for T2 and T3 tumors, respectively.[31]

Osteosarcoma

Osteosarcoma of axial sites is less common than appendicular osteosarcoma and represents approximately 25% of all cases.[109] Of the axial osteosarcomas, the mandible and maxilla are involved in 27% and 16% to 22% of cases, respectively.[109,110] The prognosis for dogs with oral osteosarcoma is better than appendicular osteosarcoma because of a lower metastatic potential.[109]

The outcome following mandibulectomy alone is variable with median survival times of 14 to 18 months and 1-year survival rates of 35% to 71%.[17,109,111] In 20 dogs treated with mandibulectomy alone, the cause of death was local recurrence in 15% of dogs and metastatic disease in 35% of dogs.[17] Following maxillectomy, the median survival time varies from 5 to 10 months with a 1-year survival rate of 17% to 27% and local tumor recurrence in 83% to 100% of dogs.[18,109] The majority of dogs with maxillary osteosarcoma die as a result of local tumor recurrence with metastasis not reported in any dog in two studies.[109,110]

Local tumor control is the most challenging problem and resecting oral osteosarcomas with complete surgical margins is imperative. In one study of 60 dogs with osteosarcoma of the skull, including the mandible and maxilla, the median disease-free interval and survival times were not reached at greater than 1503 days following complete excision and significantly better than incomplete resection with a median disease-free interval of 128 days and a median survival time of 199 days.[112] The combination of surgery with either radiation therapy or chemotherapy did not improve the outcome in dogs with incompletely resected tumors, highlighting the necessity for an aggressive surgical approach. These results are supported by another study of 45 dogs with axial osteosarcoma in which favorable prognostic factors included complete surgical excision, mandibular location, and dogs with smaller body weight.[110] The role of chemotherapy in the management of dogs with axial osteosarcoma is unknown but should be considered.

Epulides

Fibromatous and ossifying epulides

The prognosis for dogs with epulides is excellent following treatment with either surgery or radiation therapy. These are benign tumors and metastasis has not been reported, hence local tumor control is the principal goal of therapy. For fibromatous and ossifying epulides, the local tumor recurrence rate following surgical resection without bone removal varies from 0% to 17%.[55,113] Radiation therapy is also effective with a 3-year progression-free survival rate of 86%.[74] However, full-course radiation therapy is rarely required as these tumors can be adequately managed with simple surgical resection.[55] Local recurrence is common in cats with multiple epulides and is reported in 73% of 11 cats 3 months to 8 years after surgical resection.[53]

Acanthomatous epulides

Surgery or radiation therapy is used in the management of dogs with acanthomatous epulis. Mandibulectomy or maxillectomy is required for surgical resection of acanthomatous epulides because of frequent bone invasion by this benign but locally aggressive tumor. Local recurrence rates following bone-removing surgery are less than 5%.[10,12,17,18,55,57] Megavoltage radiation therapy, consisting of an alternate day protocol of 4 Gy per fraction to a total of 48 Gy, results in a 3-year progression-free survival rate of 80% in dogs with acanthomatous epulides.[74] The overall local recurrence rate varies from 8% to 18% in two studies of 39 dogs and recurrence was eight times more likely with T3 tumors compared to T1 and T2 tumors.[58,74] The majority of tumors recur within the radiation field, which suggests a higher radiation dose may be required to achieve higher rates of local tumor control, particularly for tumors greater than 4 cm in diameter.[74] Other complications

associated with radiation therapy include malignant transformation in 5% to 18% of dogs and bone necrosis in 6% of dogs.[58,74] Intralesional bleomycin has been described in four dogs and a complete response was observed in all dogs, and this response was sustained for a minimum of 1 year with no local recurrence.[114]

SELECTED SITES FOR CANCER CONDITIONS IN THE ORAL CAVITY

Tonsillar Squamous Cell Carcinoma

Tonsillar SCC is 10 times more common in animals living in urban versus rural areas, implying an etiologic association with environmental pollutants.[115] Primary tonsillar cancer is often SCC. Lymphoma can affect the tonsils but is usually accompanied by generalized lymphadenopathy and is often bilateral. Other cancers, especially malignant melanoma, can metastasize to the tonsils. Cervical lymphadenopathy is a common presenting sign, even with very small primary cancers. Fine-needle aspirates of the regional lymph nodes or excisional biopsy of the tonsil will confirm the diagnosis. Thoracic radiographs are positive for metastasis in 10% to 20% of cases at presentation. In spite of disease apparently confined to the tonsil, this disease is considered systemic at diagnosis in over 90% of cats and dogs. Simple tonsillectomy is almost never curative, but bilateral tonsillectomy should be considered due to the high percentage of bilateral disease.[9] Cervical lymphadenectomy, especially if the regional lymph nodes are large and fixed, is rarely curative and should be considered diagnostic only. Regional radiation therapy of the pharyngeal region and cervical lymph nodes is capable of controlling local disease in over 75% of the cases; however, survival still remains poor with 1-year survival rates of only 10%.[98,100] Local tumor control and survival times were significantly improved in one study of 22 dogs with tonsillar SCC when radiation therapy was combined with a variety of different chemotherapy drugs.[100] Cause of death is local disease early and systemic disease (usually lung metastasis) later. To date, no known effective chemotherapeutic agents exist for canine or feline SCC, although cisplatin, doxorubicin, vinblastine, and bleomycin have been used with limited success.[100,116]

Tongue

Cancer confined to the tongue is rare. The most common cancer of the canine tongue is SCC, accounting for approximately 50% of cases, followed by granular cell myoblastoma, malignant melanoma, mast cell tumor, fibrosarcoma, adenocarcinoma, neurofibrosarcoma, leiomyosarcoma, hemangiosarcoma and hemangioma, rhabdomyoma and rhabdomyosarcoma, myxoma, and lipoma.[117,118] Feline tongue tumors are usually SCC, and most are located on the ventral surface near the frenulum. Presenting signs are similar to other oral tumors. Ulceration is common with SCC.

Under general anesthesia, the tongue may be biopsied with a wedge incision and closed with horizontal mattress sutures. Biopsies are necessary to differentiate malignant tumors from non-neoplastic lesions such as eosinophilic granulomas and calcinosis circumscripta. Ultrasonography can be useful in delineating the margins of tongue masses to determine surgical resectability.[119] Regional lymph nodes should be aspirated for staging purposes and three-view thoracic radiographs evaluated for lung metastasis.

Surgical resection is recommended, while radiation therapy is reserved for melanomas, inoperable cancer, or tumors metastatic to the regional lymph nodes. Partial glossectomy of up to 60% of the tongue has been recommended for unilateral tumors not crossing the midline or tumors confined to the rostral mobile portion of the tongue. However, 54% of canine tongue tumors are located in the midline or are bilaterally symmetrical, which limits the ability to achieve complete surgical resection.[99] Recently, 50% to 100% resection or avulsion of the tongue was reported in five dogs with minimal postoperative problems, which suggests more aggressive resections may be possible without compromising quality of life.[120] Feeding tubes are recommended for enteral nutrition during postoperative recovery, but, in the long-term, eating and drinking are usually only mildly impaired and good hydration and nutrition can be maintained postoperatively.[117,120] Hypersalivation is the most common complaint following aggressive resections.[120] Thermoregulation can be a problem in hot and humid environments. Grooming in cats will be compromised and may result in poor hair-coat hygiene.

The prognosis for tongue tumors depends on the site, type, and grade of cancer.[117] Tongue SCCs in dogs are graded from I (least malignant) to III (most malignant) based on histologic features such as degree of differentiation and keratinization, mitotic rate, tissue and vascular invasion, nuclear pleomorphism, and scirrhous reaction.[117] The median survival time for dogs with grade I tongue SCC is 16 months following surgical resection and is significantly better than the median survival times of 4 and 3 months reported for grade II and III SCC, respectively.[117] The 1-year survival rate is 50% following complete surgical resection and approaches 80% with complete histologic resection of low-grade SCC.[117] Cancer in the rostral (mobile) tongue has a better prognosis likely because rostral lesions are detected at an earlier stage, the caudal tongue may have

richer lymphatic and vascular channels to allow metastasis, and rostral tumors are easier to resect with wide margins. Long-term control of feline tongue tumors is rarely reported with 1-year survival rates for tongue SCC less than 25%.

Granular cell myoblastoma is a curable cancer.[121] These cancers may look large and invasive but are almost always removable by conservative and close margins (Figure 21-7). Permanent local control rates exceed 80%. They may recur late but serial surgeries are usually possible. Metastasis is rare with this cancer. Local control in four of five tongue melanomas was obtained by surgery, and the metastatic rate was less than 50% in this small series.[104] In another series of dogs with tongue melanoma, the median survival time was not reached and was greater than 551 days.[6] The biologic behavior of other tongue cancers is generally unknown due to the rarity of these conditions.[104]
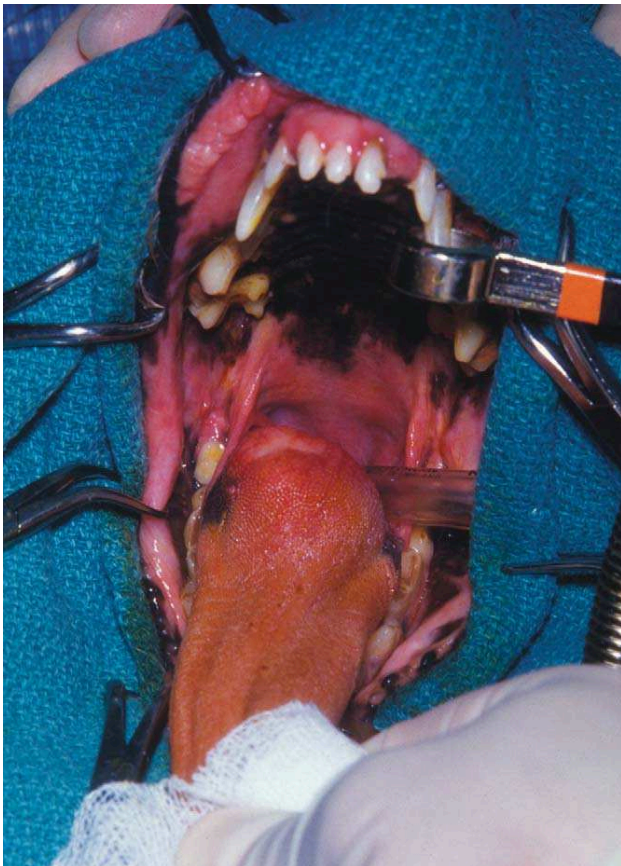
Figure 21-7

This large granular cell myoblastoma was easily removed surgically. The dog had a recurrence 2 years postoperatively, which was resected again, and the dog is tumor-free 3 years after the second surgery.

Undifferentiated Malignancy of Young Dogs

Undifferentiated malignancy is seen in dogs under 2 years of age (range, 6 to 22 months).[122] Most are large-breed dogs, and there is no sex predilection. The disease is manifest by a rapidly growing mass in the area of the hard palate, upper molar teeth, maxilla, or orbit. Biopsies reveal an undifferentiated malignancy of undetermined histogenesis. The majority of dogs present with metastasis to the regional lymph nodes and distant sites beyond the head and neck. An effective treatment has not been identified, although chemotherapy would be necessary considering the high metastatic rate. Most dogs are euthanatized within 30 days of diagnosis due to progressive and uncontrolled tumor growth.

Papillary SCC has been reported to occur in the oral cavity of very young dogs (2 to 5 months). Treatment recommendations include surgical debulking and curettage followed by radiation (40 Gy in 20 fractions). Using this combination therapy, no dog had metastasis and long-term control was achieved in all dogs for periods up to 4 years.[123]

Multilobular Osteochondrosarcoma

Multilobular osteochondrosarcoma is an infrequently diagnosed bony and cartilaginous tumor that usually arises from the canine skull, including the mandible, maxilla, orbit, and hard palate.[22,23] Histologically, these tumors are characterized by multiple lobules with a central cartilaginous or bone matrix surrounded by a thin layer of spindle cells.[22,23] On imaging, multilobular osteochondrosarcoma is characterized by a typical "popcorn ball" appearance (Figure 21-8). Surgery is recommended for management of the local tumor, although there are anecdotal reports that multilobular osteochondrosarcoma may also be responsive to radiation therapy. The overall rate of local recurrence following surgical resection is 47% to 58% and is dependent on completeness of surgical resection and histologic grade.[22,23] The median disease-free interval for completely resected multilobular osteochondrosarcoma is 1332 days and is significantly better than the 330 days reported for incompletely excised tumors.[23] The local recurrence rate for grade III tumors is 78% and significantly worse than the recurrence rates of 30% and 47% for grade I and II multilobular osteochondrosarcoma, respectively.[23] The tumor has a moderate metastatic potential, particularly to the lungs, which is grade dependent and usually occurs late in the course of disease. Metastasis is reported in up to 58% of dogs with the median time to metastasis of 426 to 542 days.[22,23] Metastasis is significantly more likely following incomplete surgical resection, with a 25% metastatic rate in completely excised tumors and 75%

Figure 21-8

A CT image of a multilobular osteochondrosarcoma of the vertical ramus of the mandible. Note the characteristic "popcorn" appearance of the mass. Following resection of the vertical ramus, this dog was tumor-free 3 years after surgery.

following incomplete resection.[23] Tumor grade also has a significant impact on metastatic rate with metastasis reported in 78% of grade III multilobular osteochondrosarcoma compared to 30% of grade I and 60% of grade II tumors.[23] There is no known effective chemotherapy treatment for metastatic disease, but survival times greater than 12 months have been reported with pulmonary metastatectomy because of the slow-growing nature of this tumor.[23] The overall median survival time is 21 months and is grade dependent, with reported median survival times of 50 months, 22 months, and 11 months for grade I, II, and III tumors, respectively.[22,23] Tumor location also has prognostic significance as the outcome for dogs with mandibular multilobular osteochondrosarcoma is significantly better with a median survival time of 1487 days compared to 587 days for these tumors at other sites.[23]

Viral Papillomatosis

Viral papillomatosis is horizontally transmitted by a DNA viral agent (papovavirus) from dog to dog.[124] Affected animals are generally young. The lesions appear wartlike and are generally multiple in the oral cavity, pharynx, tongue, or lips. A biopsy can be performed if necessary, but visual examination is usually diagnostic. Most patients never suffer any

significant side effects of this disease, while an occasional dog will have such marked involvement as to require surgical debulking in order to permit swallowing. The majority of patients will undergo a spontaneous regression of disease within 4 to 8 weeks. For resistant cases, a wide variety of treatments have been attempted, including crushing of lesions *in situ* to "release" viral antigens, autogenous vaccines, and chemotherapy using levamisole or thiabendazole.[125] However, these methods are seldom required or effective. Furthermore, autogenous vaccines are not recommended because malignant skin tumors have been reported at the site of inoculation.[54] The prognosis is usually excellent.

Odontogenic Tumors

Odontogenic tumors originate from epithelial cells of the dental lamina and account for up to 2.4% of all feline oral tumors.[2] They are broadly classified into two groups depending on whether the tumors are able to induce a stromal reaction.[126] Inductive odontogenic tumors include ameloblastic fibroma, dentinoma, and ameloblastic, complex, and compound odontomas. Ameloblastomas and calcifying epithelial odontogenic tumors are examples of noninductive odontogenic tumors.[126]

Ameloblastoma is the most common odontogenic tumor in dogs,[54] but it can be confused with acanthomatous epulis because of similar histologic features. Ameloblastomas are locally aggressive, but metastasis has not been reported.[62] Inductive fibroameloblastoma is the most common odontogenic tumor in cats, usually occurs in cats less than 18 months of age, and has a predilection for the region of the upper canine teeth and maxilla.[2,54,126-128] Radiographically the tumor site shows variable degrees of bone destruction, production, and expansion of the mandibular or maxillary bones (Figure 21-9). Teeth deformity is common. Smaller lesions are treated with surgical debulking and cryosurgery or maxillectomy/mandibulectomy. Larger lesions will respond to radiation. Local treatment needs to be aggressive, but control rates are good and metastasis has not been reported.[2,54]

Odontomas are benign tumors arising from the dental follicle during the early stages of tooth development.[129] Odontomas induce both enamel and dentin within the tumor. Odontomas have a similar biologic behavior to ameloblastomas.

Dentigerous cysts are non-neoplastic, circumscribed cystic lesions originating from islands of odontogenic epithelium.[126] They contain one or more teeth embedded in the cyst wall. Radiographs show a characteristic radiolucent halo surrounding the nonerupted tooth originating at the cementoenamel junction and enveloping the crown of the tooth.[62] Odontogenic cysts

Figure 21-9

An Intraoral radiograph of the rostral mandible in a dog with an ameloblastoma. Note the expansile mandibular mass. The tumor was curetted and filled with cancellous bone graft and the dog was tumor-free 1 year after surgery.

Figure 21-10

An extensive eosinophilic granuloma in a cat involving the lips and hard palate. These lesions can appear aggressive and similar to malignant oral tumors such as squamous cell carcinomas. An incisional biopsy is often required to differentiate this benign nonsurgical disease from malignant diseases.

may represent an early stage of malignant epithelial tumors.[126] Surgical treatment is recommended, consisting of surgical removal of nonerupted teeth and the cyst lining with possible cancellous bone grafting to prevent local tumor recurrence.[62]

Eosinophilic Granulomas

Eosinophilic granuloma in dogs

Canine oral eosinophilic granulomas affect young dogs (1 to 7 years) and may be heritable in the Siberian husky and Cavalier King Charles spaniel.[130-132] It is histologically similar to the feline disease, with eosinophils and granulomatous inflammation predominating. The granulomas typically occur on the lateral and ventral aspects of the tongue. They are raised, frequently ulcerated, and may mimic more malignant cancers in gross appearance. Treatment with corticosteroids or surgical excision is generally curative, although spontaneous regression may occur. Local recurrences are uncommon.

Eosinophilic granuloma in cats

Eosinophilic granuloma, a condition also known as rodent ulcer or indolent ulcer, occurs more commonly in female cats with a mean age of 5 years.[133-136] The etiology is unknown. Any oral site is at risk but it is most common on the upper lip near the midline (Figure 21-10). The history is usually that of a slowly progressive (months to years) erosion of the lip. Biopsies are often necessary to differentiate the condition from true cancers.

Various treatments are proposed including (in order of author preference): oral prednisone at 1 to 2 mg/kg BID for 30 days or subcutaneous methylprednisolone acetate at 20 mg/cat every 2 weeks, megestrol acetate, hypoallergenic diets, radiation therapy, surgery, immunomodulation, or cryosurgery. The prognosis for complete and permanent recovery is fair, although rare cases may undergo spontaneous regression.

Nasopharyngeal Polyps in Cats

Nasopharyngeal polyps are non-neoplastic, inflammatory masses originating from either the middle ear or eustachian tube and can extend into the external ear canal or nasopharynx.[137,138] Young cats are usually affected, with a mean age of 13.6 months in one series of 31 cats.[137,139] The cause is unknown, but a viral etiology and congenital abnormality of the branchial arches have been suggested.[138] Clinical signs include sneezing, change in voice, swallowing problems, rhinitis, and difficulty in breathing. Firm, fleshy masses can be seen or palpated in the caudal pharynx or above the soft palate. Occasionally, masses can be visualized in the external ear canal.[140,141] Radiographs or advanced imaging of the skull may reveal fluid or tissue in the tympanic bullae.[142] Traction on the stalk of the inflammatory polyp, either through the oral cavity or external ear canal depending on accessibility, is recommended for initial treatment of inflammatory polyps. However, recurrences have been reported in 50% of eight cats treated with this method alone.[137] A ventral bulla osteotomy is recommended for cats with either recurrent inflammatory polyps or evidence of tympanic bulla involvement on skull imaging. Less than 4% of cats have recurrence of inflammatory polyps following bulla osteotomy.[137,141,143]

COMPARATIVE ASPECTS[144]

Squamous cell carcinoma accounts for the vast majority of oral cancer in humans. Oral tumors are associated with alcohol and tobacco use and usually occur in patients over 40 years old. Patients with oral cancer have an increased risk of developing esophageal and lung cancer. Tumors are staged similar to animals and clinical stage influences both treatment options and prognosis.

Surgery and radiation therapy are the only options that provide the opportunity for a cure. Surgery and radiation are occasionally combined, especially when neither modality is likely to achieve a cure rate greater than 70% when used as sole therapy. Chemotherapy has a limited role for control of local disease but has shown promise, often in combination with radiation, for advanced-stage cancer.

Prognosis is strongly correlated to histologic grade, stage, and site. Metastasis, particularly to the regional lymph nodes, is more frequent with tonsillar and pharyngeal SCC and larger tumors. Tumors of the pharynx and caudal tongue are associated with a worse prognosis than cancers of the rostral tongue and oral cavity because of the higher incidence of nodal metastasis and difficulty in controlling disease once it has spread beyond the primary site.

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SECTION B
Salivary Gland Cancer

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Primary salivary gland cancer is rare in the dog and cat. Most cases are reported in older patients (10 to 12 years), and no specific breed or sex predilection has been determined in dogs.[1-3] The Siamese cat may be at higher risk than other breeds, with male cats being affected twice as often as female cats.[4]

PATHOLOGY

The vast majority of salivary cancers are adenocarcinomas, but a wide range of specific histologic types including osteosarcoma, mast cell, sebaceous carcinoma, and malignant fibrous histiocytoma has been reported.[1,5-8] They can arise from major (parotid, mandibular, sublingual, zygomatic) or minor accessory glands throughout the oral cavity. Of 245 submissions of salivary tissue for histologic diagnosis in both species, 30% were neoplastic.[3] Benign salivary gland tumors are rare in animals[9,10] compared to humans. The mandibular gland is most commonly affected.[3,4] Malignancies are variably locally invasive, and metastasis to regional lymph nodes is common (39% for cats and 17% for dogs).[4,11] Distant metastasis has been reported (16% for cats and 8% for dogs at presentation) but may be slow to develop.[4,12] Benign lipomatous infiltration of the canine salivary gland has also been reported, and surgical excision was curative in all treated cases.[13]

HISTORY AND CLINICAL SIGNS

Symptoms are nonspecific and generally include halitosis, dysphagia, exophthalmus, or a unilateral, firm, painless swelling of the upper neck (mandibular and sublingual), base of the ear (parotid), upper lip or maxilla (zygomatic), or mucous membrane of lip or tongue (accessory salivary tissue). Major differential diagnoses are mucoceles, abscesses, salivary gland infarction, sialadenitis, lymphoma, or reactive or metastatic lymphadenopathy.[3]

DIAGNOSTIC TECHNIQUES AND WORKUP

Fine-needle aspiration cytology of masses in these locations should help differentiate noncancer diseases from cancer. Regional radiographs will usually be normal but may reveal periosteal reaction on adjacent bones or displacement of surrounding structures. CT imaging may be helpful in determining extent of disease and infiltration into surrounding structures. Needle core or wedge biopsies will usually be necessary to make a definitive diagnosis.

THERAPY

When possible, aggressive surgical removal should be performed. Unfortunately, many lesions are extracapsular and widely extensive throughout the regional area, which contains numerous vital structures. However, complete extirpation of the ipsilateral neck can be performed with good functional outcome. The primary clinical impairment is the inability to blink the eye, which can be managed with a tarsorrhaphy or eye drops. If this surgery can be performed to the level of microscopic residual disease and radiation therapy is planned postoperatively, this complication is acceptable.

Postoperative radiation therapy resulted in good local control and prolonged survival in three reported cases.[6] Chemotherapy for salivary gland adenocarcinoma has been largely unreported.

PROGNOSIS

The prognosis for salivary gland cancer is generally unknown. Clinical experience on a limited number of cases would indicate that aggressive local resection (usually histologically incomplete) followed by adjuvant radiation can attain permanent local control and long-term survival.[4,14] Incomplete removal will invariably result in local recurrence.[2] Histologic grade was not prognostic for outcome, while advanced stage was a negative prognosticator.[4] Median survival for 24 dogs and 30 cats treated with surgical resection with or without adjuvant radiation was 550 and 516 days, respectively.[4] The prognosis for long-term survival for cats is worse than for dogs.[4] Another report of six dogs with salivary carcinoma treated with surgery alone had a median survival of only 74 days, and all six dogs developed pulmonary metastasis.[15]

COMPARATIVE ASPECTS[16]

Salivary gland tumors are more common in older humans than in animals and account for 4% of head and neck neoplasms. The parotid gland is most commonly affected. A wide variety of benign and malignant neoplasms are recognized. Treatment is with surgical excision, and radiation is utilized for inoperable

disease or after incomplete removal. Five-year survival usually exceeds 75% but is dependent on stage and histologic type.

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SECTION C
Esophageal Cancer

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Cancer of the esophagus is very rare and accounts for less than 0.5% of all cancer in the dog and cat.[1] Sarcomas, secondary to *Spirocerca lupi* infestation, have been reported in indigenous areas (Africa, Israel, and the southeastern United States).[2,3] No cause is known for the more common carcinomas. Most animals are older, and no sex or breed predilection is evident.

PATHOLOGY AND NATURAL BEHAVIOR

The more commonly reported histologic types include squamous cell carcinoma, leiomyosarcoma, fibrosarcoma, and osteosarcoma. Rarely, benign neoplasms such as leiomyoma and plasmacytoma may be encountered, especially in the area of the terminal esophagus and cardia.[4-6] Paraesophageal tumors such as thymic, heart base, or thyroid may also invade the esophagus.[1] In cats, squamous cell carcinomas are usually seen in females and are located in the middle third of the esophagus just caudal to the thoracic inlet.[7-10] Most esophageal cancers are locally invasive, and metastasis is to draining lymph nodes, direct extension or hematogenously to distant sites.

HISTORY AND SIGNS

Signs other than those of general debilitation and weight loss include pain on swallowing, dysphagia, and regurgitation of undigested food. Pneumonia, secondary to aspiration, may also be noted. Hypertrophic osteopathy has been reported, especially with *Spirocerca lupi*–induced sarcomas.[2] Spirocerca usually affects the caudal thoracic esophagus and can be associated with thoracic spondylitis and a microcytic hypochromic anemia and a neutrophilia.[3]

DIAGNOSTIC TECHNIQUES AND WORKUP

It is generally evident from the history that the patient is suffering from a partial or complete upper-gastrointestinal obstruction. Plain radiographs may reveal retention of gas within the esophageal lumen, a mass, or esophageal dilatation proximal to the cancer. A positive-contrast esophagogram with or without fluoroscopy will generally reveal a stricture or mass lesion in the lumen.

Esophagoscopy allows visualization of the lesion, which is frequently ulcerated. Several biopsies should be taken, since necrosis and inflammation are often prominent. The risk of esophageal perforation during the biopsy is generally minimal. Leiomyomas appear as circumscribed submucosal masses, with a normal and freely movable mucosa making endoscopic biopsy unrewarding. Occasionally, advanced imaging such as CT or MRI may be helpful to determine extent of the lesion, especially when minimal mucosal involvement is evident on endoscopic evaluation.

Open surgical biopsy via thoracotomy or cervical exploration is another option to obtain tissue for a diagnosis. *Spirocerca lupi* ova may be detected in the feces.[3]

THERAPY

Therapy for malignant cancer of the esophagus is difficult at best because of the advanced stage of disease in most cases.[11] Intrathoracic resections are further complicated by poor exposure, lengthy resections, tension on the anastomosis, and unique healing problems of the thoracic esophagus. For lesions in the caudal esophagus or cardia, gastric advancement through the diaphragm can be attempted.

Various procedures to partially replace the resected esophagus have been described, including microvascular transfer of colon or small bowel, but clinical reports on their use in the cancer patient are lacking.[12-14] Chemotherapy has rarely been attempted. Radiation therapy for the cervical esophagus can be attempted but is of limited value for the intrathoracic esophagus because of the poor tolerance of surrounding normal tissues such as the lung and heart. Short-term palliation can be achieved via esophagotomy or gastrostomy tubes. Benign leiomyomas of the esophagus or cardia can be approached via thoracotomy or celiotomy.[6]

PROGNOSIS

Except for the rare, benign lesions[4,5] or lymphoma, the prognosis is very poor for cure or palliation due to poor resection options and high metastatic rate. One report describes a series of six dogs treated with partial esophagectomy for esophageal sarcomas associated with Spirocerca. Five of the six also received doxorubicin, and the medium survival was 267 days.[15]

COMPARATIVE ASPECTS[16]

Esophageal cancer (principally squamous cell carcinoma) is rare in humans but still accounts for 7000 deaths per year in the United States. Marked geographic variance in worldwide incidence implies numerous environmental influences on development (including tobacco, alcohol, hot food, and nitrosamines).

Most esophageal cancers have extensive local tumor growth and lymph node involvement precluding curative treatment. Combinations or single use of surgery, radiation, and chemotherapy have resulted in 5-year survivals of less than 20% of the more common advanced-stage disease. A variety of palliative bypass procedures for inoperable disease are also performed (esophagogastrostomy, intraluminal intubation, dilatation, and feeding gastrostomies).

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SECTION D
Exocrine Pancreatic Cancer

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Exocrine cancer of the pancreas is very rare (<0.5% of all cancers) in the dog and uncommon in the cat.[1,2] Older female dogs and spaniels have been described as being at higher risk.[3-5] Experimentally, N-ethyl-N′-nitro-N-nitrosoguanidine has been shown to induce pancreatic duct adenocarcinoma when administered intraductally in dogs.[6]

PATHOLOGY AND NATURAL BEHAVIOR

Almost all cancers of the pancreas are epithelial and most are adenocarcinoma of ductular or acinar origin. Nodular hyperplasia is a common asymptomatic finding in older dogs or cats. "Benign" pancreatic pseudocysts and adenomas have been diagnosed by ultrasonography or surgery in dogs and cats.[2,7] In the vast majority of cases, malignant cancer has metastasized to regional or distant sites before a diagnosis can be made.[1,4]

HISTORY AND CLINICAL SIGNS

The history and clinical signs of pancreatic cancer are vague, nonspecific, and may mimic or be accompanied by pancreatitis. Weight loss and anorexia (marked in cats), paraneoplastic alopecia in cats,[8,9] vomiting, rare associated diabetes mellitus,[10] abdominal distension due to mass effect or abdominal effusions secondary to tumor implantation on the peritoneum (i.e., carcinomatosis; common in cats), icterus (with common bile duct obstruction), and depression are common symptoms. Alternatively, patients may present for symptoms of metastatic disease.

DIAGNOSTIC TECHNIQUES AND WORKUP

Most hematologic and biochemical evaluations are nonspecific but may include mild anemia, hyperglycemia, neutrophilia, and bilirubinemia (if occluding the common bile duct.).[1,2] Elevations of serum amylase and lipase are inconsistent.[11] In extreme cases, signs of pancreatic insufficiency may be exhibited.[12] Positive-contrast upper gastrointestinal radiographs may reveal slowed gastric emptying and occasionally compression or invasion of the duodenum.

Ascites may be a clinical sign and, when present, may reveal malignant cells on cytologic examination. Flow cytometry can be considered to help distinguish malignant from nonmalignant abdominal effusions. In the dog, most tumors are not palpable through the abdominal wall. In the cat, late stage, large palpable masses are often present in the author's experience.

Ultrasonography should be a useful diagnostic tool for localization of the primary tumor, documentation and aspiration of fluid as well as metastasis to liver and regional lymph nodes.[13] The utility of advanced imaging, such as CT and MRI, have not been documented for pancreatic tumors in veterinary patients; however, they will likely become more important in the future. At present, most diagnoses are made at exploratory laparotomy.

THERAPY

Most nonislet cell carcinomas of the pancreas are metastatic (regional lymph nodes and liver) or locally and extensively invasive at the time of diagnosis. If the liver, peritoneal cavity, or draining lymph nodes are positive for tumor, heroic surgery should generally not be performed. Complete pancreatectomy or pancreaticoduodenectomy (Whipple's procedure) has been described in humans and dogs,[14] but it carries a high operative morbidity and mortality without significant cure rates. Palliative gastrointestinal bypass (gastrojejunostomy) is a short-term option if bowel obstruction is imminent. Radiation and chemotherapy have shown limited value in humans and animals. Occasionally, uncomfortable effusions from carcinomatosis can be diminished with systemic or intracavitary chemotherapy (see Chapter 11); however, the palliative response tends to be short lived.

PROGNOSIS

The outlook for this disease in animals is very poor due to its critical location and advanced stage at diagnosis. One-year survival after diagnosis, regardless of treatment, has not been reported.

COMPARATIVE ASPECTS[15]

Pancreatic exocrine carcinoma accounts for more than 27,000 deaths per year in the United States. Most patients have disease progression beyond the pancreas at the time of initial diagnosis. Seventy-five percent are located in the head of the pancreas and the remainder in the body and tail. Direct extension to duodenum,

bile duct, and stomach, as well as common metastasis to lymph node and liver make treatment difficult.

When possible, pancreaticoduodenectomy (Whipple's procedure) or complete pancreatectomy are the treatments of choice. However, operative mortality ranges from 5% to 30%. Palliative bypass of the biliary tree and duodenum is commonly performed for inoperable lesions.

Traditional external beam radiation therapy is generally palliative rather than curative. Intraoperative and interstitial radiation are being explored as means for high-dose delivery to the tumor while sparing normal radiosensitive structures.[16,17] Chemotherapy alone (especially the taxols and gemcitabine) or in combination with radiation or surgery has demonstrated some improvement in quality of life yet only very modest improvements in survival. Overall, 5-year survival for all patients remains less than 5%.

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SECTION E
Gastric Cancer

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Gastric cancer is more common than esophageal cancer but still accounts for less than 1% of all malignancies. No definitive etiology is known, although long-term administration of nitrosamines may induce carcinomas in dogs.[1] Two articles have described a high incidence of gastric carcinoma in related Belgian shepherds, implying a genetic mechanism in development of this disease.[2,3] The average age of affected carcinoma patients is 8 years, with a 2.5:1 male-to-female ratio.[4,5] Males are also more commonly affected with gastric lymphoma than females.[6] Leiomyomas tend to occur in very old dogs (average age 15 years).[7,8]

PATHOLOGY AND NATURAL BEHAVIOR

Adenocarcinoma accounts for 70% to 80% of cancer of the canine stomach.[9] It is often scirrhous (firm and white serosally) and has been termed *linitis plastica* (leather bottle) because of its firm and nondistensible texture. Lesions can be diffusely infiltrative, expansile (often with a central crater and ulceration), or may look more polypoid.[10] Other reported malignancies include leiomyosarcoma,[11] lymphoma,[6] mast cell,[12] extramedullary plasmacytoma,[13,14] and fibrosarcoma. Gastrointestinal stromal tumors (GIST) have been described in the gastrointestinal tract of the dog with 20% occurring in the stomach and were likely previously classified as leiomyomas.[15] In people and in approximately 50% of cases in dogs, GIST tumors express CD117 (c-Kit), a tyrosine kinase receptor and mutations in c-Kit, likely play a role in tumorigenesis of this cancer.[15] In one series, one third of GIST in dogs had metastasized at the time of

diagnosis.[15] Adenocarcinoma will frequently spread to regional lymph nodes (70% to 80% at necropsy) followed by liver and lung.[2,5,10,12] An unusual occurrence of metastasis of gastric adenocarcinomas to the testes has been reported in three dogs.[16] Adenocarcinomas have been described as diffuse or interstitial, but little clinical significance can be associated with these variants.[5,17] Benign lesions are generally leiomyomas, hypertrophic gastropathy, or adenomas.[8,18-23] Feline gastric adenocarcinoma is rare, and the stomach is the least commonly affected gastrointestinal site in the cat.[23,24] Lymphoma is the most common gastric tumor in the cat and may be solitary or one component of systemic involvement. Most cats with gastric lymphoma are feline leukemia virus negative.

HISTORY AND CLINICAL SIGNS

The most common history is one of progressive vomiting (often blood tinged or "coffee grounds" in nature), anorexia, and weight loss. The weight loss may be a result of poor digestion, loss of protein and blood from an ulcer, or generalized tumor cachexia. Duration of symptoms is from weeks to many months.

DIAGNOSTIC TECHNIQUES AND WORKUP

Routine laboratory tests and noncontrast radiographs are generally not diagnostic. Hypoglycemia may be a paraneoplastic syndrome associated with leiomyomas or leiomyosarcomas.[25,26] A microcytic hypochromic anemia is common. Occult blood in the feces may be detected. Liver "enzymes" may be elevated due to hepatic metastasis or obstruction of the common bile duct. Thoracic radiographs are only rarely positive for metastasis at the time of initial presentation.

Positive- or double-contrast gastric radiographs may reveal a mass lesion extending into the lumen (Figure 21-11). Ulceration is also a common sign. Delayed gastric emptying, poor motility, or delayed adherence of contrast material to an ulcerated tumor may also be detected. Fluoroscopy may aid in determining motility alterations. Ultrasonography may also be a useful imaging modality in concert with fine-needle or needle core biopsy.[27,28] Malignancies tend to be sessile and adenocarcinoma tends to occur most commonly on the lesser curvature and gastric antrum (Figure 21-12). Benign lesions may be pedunculated or well circumscribed. Leiomyomas often occur at the cardia and will grow into the lumen as a smooth circumscribed mass.[8]

Gastroscopy with a flexible endoscope will generally reveal larger lesions that can be biopsied.[29,30] Several "large" samples should be taken, since most gastric
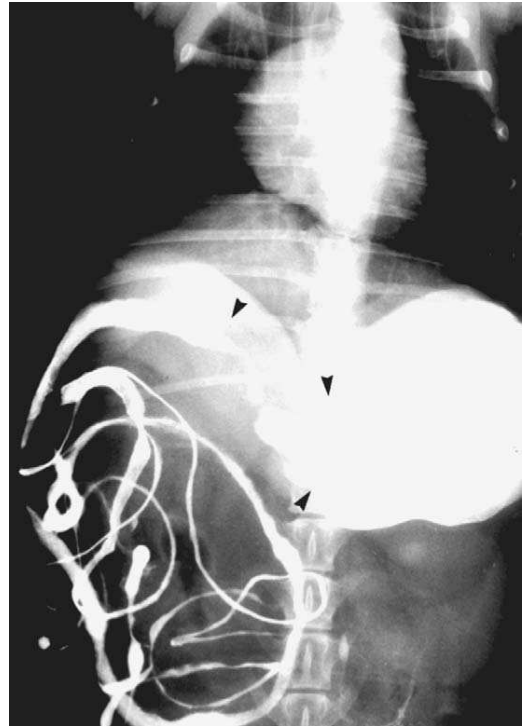
Figure 21-11

Ventrodorsal view of a dog with gastric cancer. Note the filling defect and partial outflow obstruction of gastric antrum and pylorus.

Figure 21-12

Gross specimen of the stomach from a dog with gastric adenocarcinoma. Note the large ulcer and fibrous thickening of stomach wall in area of gastric antrum (*arrows*). This patient had metastasis to regional lymph nodes and liver.

tumors have superficial necrosis, inflammation, and ulceration. In some patients, the lesions are submucosal only, making endoscopic biopsy difficult. False-negative biopsies through the gastroscope are common. Open surgical biopsy is the most definitive method of diagnosis. For suspected GIST tumors, a CD117 immunohistochemical stain should be applied for confirmation.

THERAPY

Except for lymphoma, surgery is the most common form of treatment for gastric cancer. As with esophageal cancer, curative resection is complicated by advanced-stage disease in a difficult operative area (lesser curvature, antrum, and pylorus) with a frequently debilitated patient. At the time of surgery, a careful evaluation of liver and regional lymph nodes should be made to adequately stage the cancer. Lymph node metastasis can be quite varied and all abdominal lymph nodes should be examined. If the cancer is felt to be localized to the stomach at laparotomy, a curative resection may be attempted. If possible, wide partial gastrectomy or antrectomy followed by a gastroduodenostomy (Billroth I) should be performed due to the increased morbidity associated with more extensive surgery such as gastrectomy and gastrojejunostomy (Billroth II).[31] Lesions requiring biliary bypass and very extensive surgery (complete gastrectomy) are generally too advanced to make these procedures worthwhile in terms of survival. For obstructive lesions felt to be inoperable for cure, or metastatic, it is possible to perform a palliative gastrojejunostomy to allow passage of food into the intestine, although this procedure maybe associated with significant postoperative mobidity.[31]

Leiomyomas are usually discrete, solitary lesions in the area of the cardia. They are not premalignant but can cause symptoms because of a mass effect. They can be easily "shelled out" via a midline laparotomy, gastrotomy, and submucosal removal or via intercostal thoracotomy if the side of the lesion can be clearly delineated.[8] In people, GIST tumors may be responsive to inhibitors of c-Kit (e.g., imatinib), and if safe and inexpensive inhibitors become available in veterinary-based oncology, they could theoretically have efficacy for this tumor.

Radiation therapy is rarely utilized due to the poor radiation tolerance of surrounding normal tissue (liver and intestine). Nonresectable lymphoma may be dramatically reduced with lower doses of irradiation than required for other tumors. No effective chemotherapy is known for adenocarcinoma.

Lymphoma may be excised if localized but does not generally respond well to conventional chemotherapy.[6,32] The need for postoperative chemotherapy after "complete" resection of lymphoma is unknown.

PROGNOSIS

The prognosis for most malignant gastric cancer is poor. Even if surgery can be performed, most patients are dead within 6 months as a result of recurrent or metastatic cancer.[3,9,33-37] Few adenocarcinoma patients are operable for cure, and the short-term morbidity with radical resection can be high. Of 17 dogs treated with surgery for gastric adenocarcinoma, the median survival was 2 months.[9] Rare cases have survived as long as 3 years.[3] Palliation via bypass can be achieved for 1 to 6 months. The median survival for seven dogs with gastric leiomyosarcoma that lived at least 2 weeks postoperatively was 1 year.[11] The hypoglycemia seen with some smooth muscle tumors is reversible after tumor resection. Patients with benign lesions can be cured with complete surgical excision.[8,38,39] Gastric extramedullary plasmacytomas appear to carry an excellent prognosis with surgery and chemotherapy. Gastrointestinal mast cell tumors were usually metastatic to regional nodes, and fewer than 10% survived 6 months.[12,13,14]

COMPARATIVE ASPECTS[40]

Gastric cancer is the sixth most common cause of cancer death in humans. Adenocarcinoma constitutes over 90% of all malignant gastric cancer. Multiple socioeconomic, geographic, and environmental factors are associated with risk of tumor development. *Helicobacter* has been associated with development of human gastric carcinoma but has not been correlated with carcinogenesis in the dog.

Most lesions will be firm, ulcerative, and located in the antrum or lower third of the stomach, as in the dog. Most lesions are detected late in the course of disease and have direct tumor extension to surrounding organs, lymph node metastasis, or systemic metastasis, as with the dog.

Treatment is with surgical resection when possible or with less effective radiation and chemotherapy. Five-year survival for all patients is less than 10%, with a 30% survival for patients deemed operatively to have "localized" disease.

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SECTION F
Hepatobiliary Tumors

Julius M. Liptak

INCIDENCE AND RISK FACTORS

Primary hepatic tumors are uncommon and account for less than 1.5% of all canine tumors and 1.0% to 2.9% of all feline tumors but up to 6.9% of nonhematopoietic tumors in cats.[1-4] Metastasis to the liver from nonhepatic neoplasia is more common and occurs 2.5 times more frequently than primary liver tumors in dogs, particularly from primary cancer of the spleen, pancreas, and gastrointestinal tract.[1,2] Primary hepatobiliary tumors are more common than metastatic disease in cats.[4] The liver can also be involved in other malignant processes, such as lymphoma, malignant histiocytosis, and systemic mastocytosis.[2,3] Nodular hyperplasia is a relatively common diagnosis in older dogs but is benign and probably does not represent a preneoplastic lesion.[4]

There are four basic histologic categories of primary malignant hepatobiliary tumors in cats and dogs:

TABLE 21-7	Morphologic Types of Canine Hepatic Tumors		
	Nodular	**Massive**	**Diffuse**
Hepatocellular Carcinoma	16–25%	53–84%	0–19%
Biliary Carcinoma	0–21%	37–46%	17–54%
Carcinoid	33%	0%	67%
Sarcoma	64%	36%	0%

hepatocellular, bile duct, neuroendocrine (or carcinoid), and mesenchymal (sarcoma).[4] Malignant tumors are more common in dogs, while benign tumors occur more frequently in cats.[2-8] There are three morphologic types of these primary hepatic tumors: massive, nodular, and diffuse.[5] The relative frequency of morphologic types within histologic catagories are listed in Table 21-7.[2,5,9-14,24] Massive liver tumors are defined as a large, solitary mass confined to a single liver lobe (Figure 21-13); nodular tumors are multifocal and involve several liver lobes (Figure 21-14); and diffuse involvement may represent the final spectrum of neoplastic disease with multifocal or coalescing nodules in all liver lobes or diffuse effacement of the hepatic parenchyma (Figure 21-15).[4,5]

The prognosis for cats and dogs with liver tumors is determined by histology and morphology. The prognosis is good for massive hepatocellular carcinoma (HCC) and benign tumors as complete surgical resection is possible and their biologic behavior is relatively nonaggressive.[7-11] In contrast, the prognosis is poor for cats with any type of malignant tumor, dogs with malignant tumors other than massive HCC, and cats and dogs with nodular and diffuse liver tumors as metastasis is more common and resection is not feasible.[2-14]

Figure 21-14

Necropsy specimen of a bile duct carcinoma from a cat illustrating the nodular morphology.

PATHOLOGY AND NATURAL BEHAVIOR

Hepatocellular Tumors

Hepatocellular tumors include HCC, hepatocellular adenoma (or hepatoma) and hepatoblastoma.[4] Hepatoblastoma is a rare tumor of primordial hepatic stem cells and has only been reported in one dog.[15] Hepatocellular adenoma is usually an incidental finding and rarely causes clinical signs.[2] Of the hepatocellular tumors, hepatocellular adenoma is more common in cats, and HCC occurs more frequently in dogs.[2,5,6]

Hepatocellular carcinoma is the most common primary liver tumor in dogs, accounting for 50% of

Figure 21-13

A solitary hepatic leiomyosarcoma from a dog with classic massive liver tumor morphology.
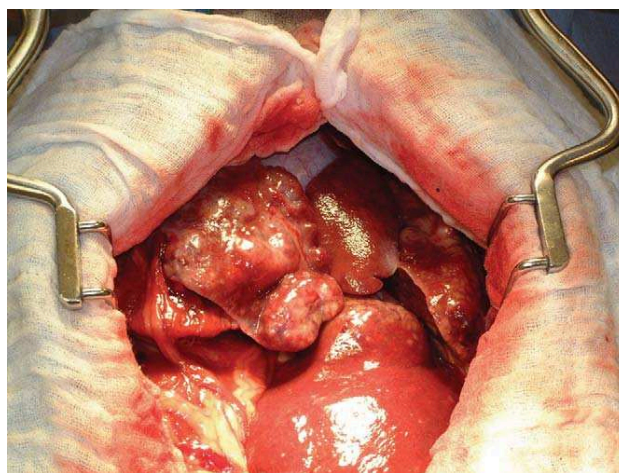
Figure 21-15

A dog with a bile duct carcinoma of diffuse morphologic appearance.

cases, and second most common in cats.[2-8] Etiologic factors implicated in the development of HCC in humans include infection with hepatitis viruses B or C, and cirrhosis.[16] A viral etiology has also been demonstrated in woodchucks, but not in cats or dogs, and cirrhosis is rare in dogs with HCC.[6-9] In one study, 20% of dogs with HCC were diagnosed with additional tumors although most were benign and endocrine in origin.[5] A breed and sex predisposition has not been confirmed in dogs with HCC, but miniature schnauzers and male dogs are overrepresented in some studies.[5,9,11,17] Metastasis to regional lymph nodes, peritoneum, and lungs is more common in dogs with nodular and diffuse HCC.[2,5,9] Other metastatic sites include the heart, kidneys, adrenal gland, pancreas, intestines, spleen, and urinary bladder.[2,5,9] The metastatic rate varies from 0% to 37% for dogs with massive HCC and 93% to 100% for dogs with nodular and diffuse HCC.[2,5-11]

Bile Duct Tumors

Bile duct adenoma (biliary cystadenoma)
There are two types of bile duct tumors in cats and dogs: bile duct adenoma and carcinoma.[2,5-8,12,13,18-22] Bile duct adenomas are common in cats, accounting for more than 50% of all feline hepatobiliary tumors, and are also known as biliary or hepatobiliary cystadenomas due to their cystic appearance (Figure 21-16).[6-8,18-20] Male cats may be predisposed.[18,20] Bile duct adenomas
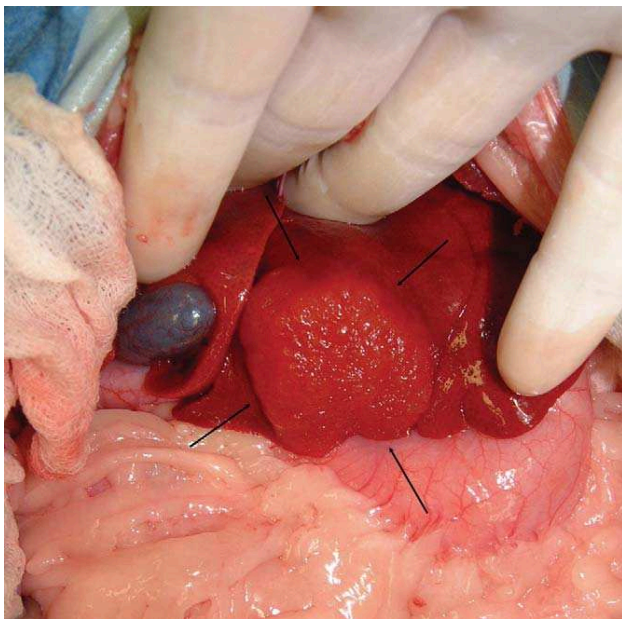
Figure 21-16
Intraoperative appearance of a bile duct adenoma in a cat. Surgical resection was curative in this cat.

usually do not cause clinical signs until they reach a large size and compress adjacent organs.[18-20] There is an even distribution between single and multiple lesions.[6-8,18-20] Malignant transformation has been reported in humans and anaplastic changes have been observed in some feline adenomas.[6,18]

Bile duct carcinoma (cholangiocarcinoma)
Bile duct carcinoma is the most common malignant hepatobiliary tumor in cats and the second most common in dogs.[2,5-8] Bile duct carcinomas account for 22% to 41% of all malignant liver tumors in dogs.[5,23] In humans, trematode infestation, cholelithiasis, and sclerosing cholangitis are known risk factors for bile duct carcinoma.[24] Trematodes may also be involved in the etiology of bile duct carcinoma in cats and dogs, but they are unlikely to be a major contributor because bile duct carcinomas also occur in geographic regions outside the normal distribution of trematodes.[4,8,13]

A predilection for Labrador retrievers has been proposed.[13] A female sex predisposition has been reported in dogs.[5,12,17] In cats, sex predisposition is conflicting with both male and female cats reported to be predisposed.[6-8] Bile duct carcinomas can be intrahepatic, extrahepatic, or within the gallbladder.[2-5-8,12,13] Intrahepatic carcinomas are more common in dogs,[5,12,13] while an equal distribution of intra- and extrahepatic tumors to extrahepatic predominance has been reported in cats.[6-8] Solid and cystic (or cystadenocarcinoma) bile duct carcinomas have been reported but this distinction does not influence either treatment or prognosis.[12] Bile duct carcinoma of the gallbladder is rare in both species.[2,5-8,12,13]

Bile duct carcinomas have an aggressive biologic behavior. Metastasis is common in dogs with up to 88% metastasizing to the regional lymph nodes and lungs (Figure 21-17), with other sites including the heart, spleen, adrenal glands, pancreas, kidneys, and spinal cord.[2,5,12,13] In cats, diffuse intraperitoneal metastasis and carcinomatosis occurs in 67% to 80% of cases.[6-8]

Neuroendocrine Tumors

Neuroendocrine tumors, also known as carcinoids, are rare in cats and dogs.[2,5-8] These tumors arise from neuroectodermal cells and are histologically differentiated from carcinomas with the use of silver stains.[3,14] Neuroendocrine hepatobiliary tumors are usually intrahepatic, although extrahepatic tumors have been reported in the gallbladder.[14,21,22] Carcinoids tend to occur at a younger age than other primary hepatobiliary tumors.[5,14] Primary hepatic neuroendocrine tumors have an aggressive biologic behavior with frequent involvement of more than one liver lobe and metastasis to the regional lymph nodes, peritoneum, and lungs in 93% of dogs.[5,14] Other metastatic sites include heart, spleen, kidneys, adrenal glands, and pancreas.[14]
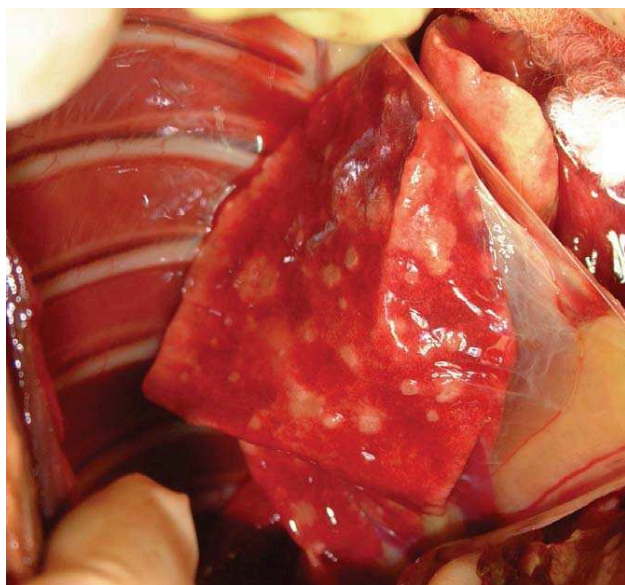
Figure 21-17

Lung metastasis in the cat with bile duct carcinoma depicted in Figure 21-14. This cat also had diffuse peritoneal metastasis.

Sarcomas

Primary and nonhematopoietic hepatic sarcomas are rare in cats and dogs.[2,5-8,24] The most common primary hepatic sarcomas are hemangiosarcoma (HSA), leiomyosarcoma (see Figure 21-13), and fibrosarcoma, with HSA the most frequently diagnosed primary hepatic sarcoma in cats and leiomyosarcoma the most common in dogs.[2,5-8,24-28] The liver is a common site for metastatic HSA in dogs while only 4% to 6% of HSA occur primarily in the liver.[27,28] Other primary hepatic sarcomas include rhabdomyosarcoma, liposarcoma, osteosarcoma, and malignant mesenchymoma.[2-8] The liver, with lungs, lymph nodes, spleen, and bone marrow, is commonly involved in dogs with disseminated histiocytic sarcoma.[29,30] Benign mesenchymal tumors, such as hemangiomas, are rare.[2-8] There are no known breed predispositions although a male predilection has been reported.[5] Hepatic sarcomas have an aggressive biologic behavior with metastasis to the spleen and lungs reported in 86% to 100% dogs.[5,24]

Other Primary Hepatic Tumors

Myelolipoma is a benign hepatobiliary tumor in cats.[3,4] Histologically, myelolipomas are composed of well-differentiated adipose tissue intermixed with normal hematopoietic elements.[4] Chronic hypoxia has been proposed an etiologic factor as myelolipomas have been reported in liver lobes entrapped in diaphragmatic herniae.[4] Myelolipomas can either be single or multifocal.[4]

HISTORY AND PHYSICAL FINDINGS

Hepatobiliary tumors are symptomatic in approximately 50% of cats and 75% of dogs, especially in animals with malignant tumors.[1-15] The most common presenting signs are nonspecific, such as inappetence, weight loss, lethargy, vomiting, polydipsia-polyuria, and ascites.[1-15] Weakness, ataxia, and seizures are uncommon and may be caused by hepatic encephalopathy, paraneoplastic hypoglycemia, or central nervous system metastasis.[5,9,31] Icterus is more common in dogs with extrahepatic bile duct carcinomas and diffuse neuroendocrine tumors.[2,5,12] However, these symptoms rarely assist in differentiating primary and metastatic liver tumors from non-neoplastic hepatic diseases.[3] Physical examination findings can be equally unrewarding. A cranial abdominal mass is palpable in up to three quarters of cats and dogs with liver tumors, although palpation can be misleading as hepatic enlargement may either be absent in nodular and diffuse forms of liver tumors, or missed due to the location of the liver in the cranial abdominal cavity deep to the costal arch.[1-15]

STAGING AND DIAGNOSIS

Laboratory Tests

Hematologic and serum biochemical abnormalities are usually nonspecific. Leukocytosis, anemia, and thrombocytosis are common in dogs with liver tumors.[1-14] Leukocytosis is probably caused by inflammation and necrosis associated with large liver masses.[9,10] Anemia is usually mild and nonregenerative.[5,11] The cause of anemia is unknown, although anemia of chronic disease, inflammation, red blood cell sequestration, and iron deficiency may be involved.[32] Thrombocytosis, defined as a platelet count greater than $500 \times 10^3/\mu L$, is seen in approximately half of dogs with massive HCC.[11] Proposed causes of thrombocytosis include anemia, iron deficiency, inflammatory cytokines, and paraneoplastic production of thrombopoietin.[33-35] Anemia and thrombocytopenia are relatively common in dogs with primary and metastatic hepatic HSA.[3] Prolonged coagulation times (e.g., increased prothrombin time, thrombin time, and activated partial thromboplastin time) and specific clotting factor abnormalities (e.g., decreased factor VIII:C and increased factor VIII:RA and fibrinogen degradation products) have been identified in dogs with hepatobiliary tumors, although these are rarely clinically relevant.[36]

Liver enzymes are commonly elevated in dogs with hepatobiliary tumors (Table 21-8). Increased activity of liver enzymes probably reflects hepatocellular damage or biliary stasis and are not specific for hepatic neoplasia.[4] There is also no correlation between the degree of

TABLE 21-8	Common Clinicopathologic Abnormalities in Dogs and Cats with Hepatobiliary Neoplasia	
Parameter	**Dogs**	**Cats**
↑ SAP	61–100%	10–64%
↑ ALT	44–75%	10–78%
↑ AST	56–100%	15–78%
↑ GGT	39%	78%
↑ LDH	36–61%	
↑ Tbili	18–33%	33–78%
↑ SBA	50–75%	67%
↓ PVC	27–50%	
↑ WBC	54–73%	
↓ Albumin	52–83%	

SAP, Alkaline phosphatase. ALT, Alanine aminotransferase. AST, Aspartate aminotransferase. GGT, Gamma-glutamyl-transferase. LDH, Lactate dehydrogenase. Tbili, Total bilirubin. SBA, Serum bile acids. PCV, Packed cell volume. WBC, Total white blood cell count.

hepatic involvement and magnitude of liver enzyme alterations.[4,11] The type of liver enzyme abnormalities may indicate the type of tumor and differentiate primary and metastatic liver tumors.[37] Alkaline phosphatase (ALP) and alanine transferase (ALT) are commonly increased in dogs with primary hepatic tumors, while aspartate aminotransferase (AST) and bilirubin are more consistently elevated in dogs with metastatic liver tumors.[1,38] Furthermore, an AST-to-ALT ratio less than 1 is consistent with HCC or bile duct carcinoma, while a neuroendocrine tumor or sarcoma is more likely when the ratio greater than 1.[5] In general, however, liver enzyme elevations are not specific for the diagnosis of hepatobiliary diseases.[39] Other changes in the serum biochemical profile in dogs with hepatic tumors may include hypoglycemia, hypoalbuminemia, hyperglobulinemia, and increased pre- and postprandial bile acids.[1,2,5,9-14] In contrast to dogs, azotemia is often present in cats with hepatobiliary tumors and may be the only biochemical abnormality, although liver enzyme abnormalities, especially elevated ALT, AST and total bilirubin are also common and are significantly higher in cats with malignant tumors.[6-8]

Alpha-fetoprotein, an oncofetal glycoprotein, is used in the diagnosis, monitoring response to treatment, and prognostication of HCC in humans.[16] In dogs, serum levels of α-fetoprotein are increased in 75% of HCC and 55% of bile duct carcinomas.[40,41] However, α-fetoprotein has limited value in the diagnosis and treatment monitoring of canine HCC as serum levels of α-fetoprotein are also increased in other types of liver tumors, such as bile duct carcinoma and lymphoma, and non-neoplastic hepatic disease.[41,42]

Imaging

Radiographs, ultrasonography, computed tomography (CT), and magnetic resonance (MR) imaging can be used for the diagnosis, staging, and surgical planning of cats and dogs with hepatobiliary tumors. A cranial abdominal mass, with caudal and lateral displacement of the stomach, is frequently noted on abdominal radiographs of cats and dogs with massive liver tumors.[10,11,17] Mineralization of the biliary tree is a rare finding in dogs with bile duct carcinoma.[4] Sonographic examination is recommended as these radiographic findings are not specific for the diagnosis of a hepatic mass and do not provide information on the relationship of the hepatic mass with regional anatomic structures.

Abdominal ultrasonography is the preferred method for identifying and characterizing hepatobiliary tumors in cats and dogs;[20,43-47] however, as the veterinary use of CT/MR imaging expands, these tools may prove more useful. Sonographic examination is useful in determining the presence of a hepatic mass and defining the tumor as massive, nodular, or diffuse.[20,43-47] If focal, the size and location of the mass, and its relationship with adjacent anatomic structures, such as the gallbladder and caudal vena cava, can be assessed.[20,43-47] Tumor vascularization can be determined using Doppler imaging techniques.[4] The ultrasonographic appearance of hepatobiliary tumors varies and does not correlate with histologic tumor type.[20,43-47]

Ultrasound-guided fine-needle aspiration or needle-core biopsy of hepatic masses is a useful, minimally invasive technique to obtain cellular or tissue samples for diagnostic purposes.[44-47] A coagulation profile is recommended prior to hepatic biopsy because mild to moderate hemorrhage is the most frequent complication, occurring in approximately 5% of cases.[44-47] A correct diagnosis is obtained in up to 60% of hepatic aspirates and 90% of needle-core biopsies.[44-48] More invasive techniques, such as laparoscopy and open keyhole approaches, can also be used for the biopsy and staging of cats and dogs with suspected liver tumors. In humans, laparoscopy is recommended for local staging as up to 20% of cases do not proceed with open surgery because of either nodular or diffuse tumor morphology or unresectable disease.[49] However, for solitary and massive hepatic masses, surgical resection can be performed without a preoperative biopsy as both diagnosis and treatment can be achieved in a single procedure.

Advanced imaging techniques, such as CT and MRI, are preferred in humans for the diagnosis and staging of liver tumors.[16] Unlike ultrasonography, imaging appearance may provide an indication of tumor type.[16] Furthermore, CT and MR are more sensitive for the detection of small hepatic lesions and determining the relationship of liver masses with adjacent vascular and soft tissue structures.[16]

Imaging is also important for the staging of cats and dogs with liver tumors. Local extension and regional metastasis can be assessed with abdominal ultrasonography, CT, MRI, or laparoscopy. *The sonographic, and sometimes gross, appearance of nodular hyperplasia and metastatic disease is similar.* In two studies, 25% to 36% of dogs with ultrasonographically detectable focal hepatic lesions were diagnosed with nodular hyperplasia.[44,50] Biopsy of such lesions is recommended prior to definitively diagnosing metastatic disease and excluding animals from curative-intent surgery.[51] Although rare at the time of diagnosis, three-view thoracic radiographs or advanced imaging techniques should be assessed prior to treatment for evidence of lung metastasis.

TREATMENT AND PROGNOSIS

Hepatocellular Tumors

Liver lobectomy is recommended for cats and dogs with a hepatic tumor that has a massive morphologic appearance, particularly HCC. Surgical techniques for liver lobectomy include finger-fracture, mass ligation, mattress sutures, and surgical stapling.[52] Mass ligation is not recommended for large dogs, tumors involving either the central or right liver divisions, or tumors with a wide base.[52] The finger-fracture technique, involving blunt dissection through hepatic parenchyma and individual ligation of bile ducts and vessels, is acceptable for smaller lesions. Surgical staplers are preferred for liver lobectomy as operative time is shorter with fewer complications (Figure 21-18).[52] Advanced imaging and intraoperative ultrasonography may provide information on the relationship of right-sided and central liver tumors with the caudal vena cava prior to liver lobectomy. In one report of 42 dogs with massive HCC

treated with liver lobectomy, the intraoperative mortality rate was 4.8% and the complication rate was 28.6%.[11] Complications include hemorrhage, vascular compromise to adjacent liver lobes, and transient hypoglycemia and reduced hepatic function.[4,11,52]

Prognostic factors in dogs with massive HCC include surgical treatment, side of liver involvement, ALT and AST activity, and ratios of ALP-to-AST and ALT-to-AST.[11] The median survival time for 42 dogs with massive HCC following liver lobectomy was not reached and >1460 days because the majority of dogs were either still alive or died of diseases unrelated to their liver tumor (Figure 21-19).[11] In comparison, the median survival time of 270 days was significantly decreased for six dogs managed conservatively and these dogs were 15.4 times more likely to die of tumor-related causes than dogs treated surgically.[11] Right-sided liver tumors, involving either the right lateral lobe or caudate process of the caudate lobe, had a poorer prognosis as intraoperative death was more likely due to caudal vena cava trauma during surgical dissection.[11] There was no difference in survival time if dogs with right-sided massive HCC survived surgery.[11] Increased ALT and AST were associated with a poor prognosis, and this may reflect more severe hepatocellular injury secondary to either large tumor size or more aggressive biologic behavior.[11]

The prognosis for dogs with massive HCC is good. Local tumor recurrence is reported in 0% to 13% of dogs with massive HCC following liver lobectomy.[10,11]

Figure 21-18

Liver lobectomy of a massive hepatocellular carcinoma using a thoracoabdominal surgical stapling device.
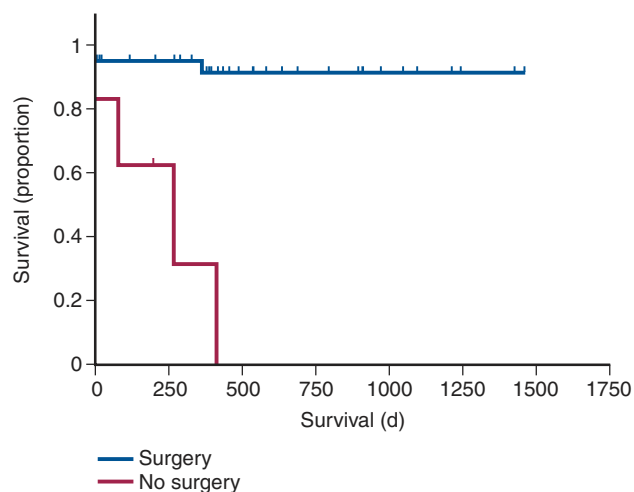
Figure 21-19

Kaplan-Meier survival curve for dogs with massive hepatocellular carcinoma. The median survival time for dogs with surgically resected tumors is significantly better than it is for dogs not treated with curative-intent liver lobectomy. *(Reprinted with permission from J. M. Liptak, W. S. Dernell, E. Monnet, et al., "Massive hepatocellular carcinoma in dogs: 48 cases (1992–2002)." J Am Vet Med Assoc 225:1225, 2004.)*

Metastasis to the liver and lungs has been documented in 0% to 37% of dogs, but metastasis is rare in recent clinical reports and most deaths are unrelated to HCC.[5,10,11]

In contrast, the prognosis for dogs with nodular and diffuse HCC is poor. Surgical resection is usually not possible due to involvement of multiple liver lobes. Treatment options for nodular and diffuse HCC in humans include liver transplantation or minimally invasive procedures for regional control, such as ablation or embolization.[16] Bland embolization and chemoembolization have been reported with moderate success in the palliation of four dogs with HCC.[53,54] The role of radiation and chemotherapy in the management of HCC is unknown. Radiation therapy is unlikely to be effective as the canine liver cannot tolerate cumulative doses greater than 30 Gy.[4,16] Hepatocellular carcinoma is considered chemoresistant in humans as response rates are usually less than 20%.[4,16] The poor response to systemic chemotherapy is probably caused by rapid development of drug resistance, either due to the role of hepatocytes in detoxicification or expression of P-glycoprotein, a cell membrane efflux pump associated with multidrug resistance.[4] Chemotherapy has not been investigated in dogs with HCC. Novel treatment options currently being investigated in human medicine include immunotherapy, hormonal therapy with tamoxifen, and antiangiogenic agents.[16]

Bile Duct Tumors

Bile duct adenomas can present as either single (e.g., massive) or multifocal lesions. Liver lobectomy is recommended for cats with single bile duct adenoma or multifocal lesions confined to one to two lobes.[6-8,18-20] The prognosis is very good following surgical resection with resolution of clinical signs and no reports of local recurrence or malignant transformation.[8,18,19]

Liver lobectomy is also recommended for cats and dogs with massive bile duct carcinoma. However, survival time has been poor in cats and dogs treated with liver lobectomy as the majority died within 6 months due to local recurrence and metastatic disease.[8,55] There is no known effective treatment for cats and dogs with nodular or diffuse bile duct carcinomas as these lesions are not amenable to surgical resection and other treatments are often not successful.

Neuroendocrine Tumors

Carcinoids have an aggressive biologic behavior and are usually not amenable to surgical resection as solitary lesions and massive morphology is rare.[5,14] The efficacy of radiation therapy and chemotherapy is unknown. Prognosis is poor as metastasis to the regional lymph nodes, peritoneum, and lungs occurs in 93% of dogs and usually early in the course of disease.[5,14]

Sarcomas

Liver lobectomy can be attempted for solitary and massive sarcomas. However, prognosis is poor as metastatic disease is often present at the time of surgery.[5,24] Chemotherapy has not been investigated in the treatment of primary hepatic sarcomas although, similar to other solid sarcomas, response rates are likely to be poor. Doxorubicin-based protocols and ifosfamide have shown some promise with sarcomas in other locations and warrant consideration for cats and dogs with primary hepatic sarcomas.[56,57]

Other Primary Hepatic Tumors

Surgical resection with liver lobectomy is recommended for cats with primary hepatic myelolipoma and the prognosis is excellent with prolonged survival time and no reports of local recurrence.[4]

COMPARATIVE ASPECTS

Hepatocellular carcinoma is one of the most common malignancies in humans as a result of viral infections with hepatitis viruses B and C, and cirrhosis induced by alcohol consumption and other disease.[16] A number of paraneoplastic syndromes have been described including hypoglycemia, erythrocytosis, and hypercalcemia.[16] Ultrasonography is considered a good screening imaging modality, but advanced imaging with contrast-enhanced CT or MRI is preferred to determine the location, size, and extent of hepatic lesions.[16] Other tests include serum α-fetoprotein, serologic tests for hepatitis B and C viruses, and histologic confirmation with core liver biopsies.[16] Unlike HCC in dogs, the morphology of HCC in humans is often nodular or diffuse which makes definitive treatment more problematic. Treatment options depend on the stage of disease and include surgery (e.g., liver lobectomy and liver transplantation), local ablative therapies (e.g., cryosurgery, ethanol or acetic acid injection, and microwave or radiofrequency ablation), regional therapies (e.g., transarterial chemotherapy, embolization, chemoembolization, or radiotherapy), and systemic treatment with chemotherapy or immunotherapy.[16] Response rates to single- and multiple-agent chemotherapy protocols are less than 25%, and chemotherapy is no longer recommended for human patients with HCC.[16]

Bile duct carcinomas are rare and, similar to cats and dogs, often associated with a poor prognosis.[25] Risk factors include primary sclerosing cholangitis, the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* in endemic areas of Southeast Asia and China, and cholelithiasis.[25] Surgical resection is preferred, but, because of the high rate of local or regional recurrence, adjuvant treatment with radiation therapy or chemotherapy is recommended.[25] However, because of the rarity of

this tumor, studies supporting the efficacy of these adjuvant treatments are lacking. Papillary histology, extrahepatic location, and complete resection are favorable prognostic factors in humans with bile duct carcinomas.[58]

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SECTION G
Intestinal Tumors

Kim A. Selting

INCIDENCE

Intestinal tumors account for less than 10% of all tumors in dogs and cats.[1-3] In one report from the United Kingdom, intestinal tumors accounted for 22% of alimentary tumors in dogs and 35% in cats.[1] Less than 1% of more than 10,000 dogs submitted for necropsy at one institution were diagnosed with intestinal adenocarcinoma.[4,5] Lymphoma comprises nearly 30% of all feline tumors and 6% of all canine tumors, and is the most common intestinal tumor in most reports.[2,6] In a survey of insured dogs in the United Kingdom, an incidence rate of 210/100,000 dogs was reported for alimentary tumors, which accounts for 8% of all tumor submissions.[7] In a South African survey, feline intestinal neoplasia comprised 13.5% of all tumors.[8] Proportion of intestinal to other types of tumors in non-U.S. countries may be skewed by cultural and environmental factors, including neutering practices.

A survey of the online Veterinary Cancer Registry identified approximately 74% of reported feline small intestinal tumors as lymphoma, which is consistent with previous reports. Adenocarcinoma accounted for 17% and other tumor types reported included mast cell tumor and leiomyosarcoma. Intestinal tumors accounted for 6% of all submitted feline tumors. A similar search in dogs found 29% of reported tumors to be lymphoma, an equal number of carcinoma cases, and 23% leiomyosarcoma, with small intestinal tumors comprising just over 1% of all tumor submissions.[9] Other studies have also shown a slight increase in adenocarcinoma incidence as compared to leiomyosarcoma.[10]

RISK FACTORS

Incidence of intestinal neoplasia increases in older dogs and cats. Mean ages generally range between 10 and 12 years for cats and 6 to 9 years in dogs.[2,10-23] The mean age for dogs with leiomyosarcoma may be older (12 years). Earlier studies of feline lymphoma report younger median ages, most likely a result of a larger percentage of FeLV positive cats in the study population.[24,25]

A slight sex predilection for males is reported for both dogs and cats.[23,26-29] Males appear overrepresented in smooth muscle tumors, constituting 82% of gastrointestinal leiomyomas[30] and 76% of dogs with leiomyosarcoma.[22] Additionally, 90% of dogs with gastrointestinal lymphoma were male.[21] Furthermore, there is a slight male predominance in nonlymphomatous small intestinal tumors in dogs.[10,31,32]

In cats, a predominance of males are reported in some studies[16,33] while not in others.[11,12,14,15,17-19,24,25,34-36] Three of four cats reported with large granular lymphoma were female.[13]

Siamese cats are overrepresented (up to eight-fold) in studies of intestinal adenocarcinoma and lymphoma, suggesting a predisposition for theses diseases.[2,12,14,31,37] Regarding dogs, large breeds in general constituted most cases in a series of smooth muscle tumors and one series observed a significant overrepresentation of collies and German sheperd dogs for intestinal tumors, especially adenocarcinoma.[20,27] Interestingly, in 104 benign and malignant tumors diagnosed in a cohort of military working dogs (German sheperd dogs and Belgian Malinois), only one (a leiomyosarcoma) was intestinal.[38]

With the exception of retroviral influence on the development of feline lymphoma, there are no known etiologic organisms or chemical agents that reliably contribute to the development of spontaneously occurring intestinal neoplasia in dogs and cats. In addition to the known association of feline leukemia virus (FeLV) and

feline immunodeficiency virus (FIV) with feline lymphoma, the link between FeLV and the intestinal form of feline lymphoma has been examined. While previous reports show that older cats with intestinal lymphoma are usually negative for FeLV on serology, evaluation of feline lymphoma by polymerase chain reaction (PCR) and immunohistochemistry (IHC) has shown a percentage of tumors to be positive for viral DNA, with young cats more likely to be positive by IHC.[33,39,40]

In one study, of feline lymphoma (both nonintestinal and intestinal), 23% of all cases tested with serology, PCR, and IHC had positive PCR and negative serology for FeLV. Overall, 80% of samples were positive on PCR. These results suggest latent/occult FeLV exposure may be involved in the development of lymphoma The role of FeLV in the induction of feline lymphoma in a serologically negative cat may result from latent, replication-deficient, or partial genome virus infection, or from a "hit and run" in which the virus is not incorporated into the host genome.[39] There is no known association of retroviral infection with nonlymphomatous intestinal neoplasia in cats.[14,31]

In reports in other species, type-A retrovirus particles have been found in a metastatic intestinal adenocarcinoma in a boa, and cytomegalovirus has been associated with gastrointestinal epithelial masses in macaque monkeys infected with simian immunodeficiency virus.[41,42]

While *Helicobacter pylori* infection is associated with increased risk of gastric cancer in people, no such association has been confirmed in domestic animals. Concurrent lymphoma and *Helicobacter* infection has been reported in a cat, but cause and effect has not been established.[43] Multiple gastroduodenal adenocarcinomas and a rectal adenoma were found in a cougar with concurrent *Helicobacter*-like organisms and spirochetes, but no causality was established.[44] Some cats shed *Helicobacter* species in the feces and thus may represent normal flora rather than pathogens.[45]

A significant increase in cats presenting to a veterinary medical teaching hospital was reported in the post-FeLV vaccine era and was due primarily to an increase in intestinal lymphoma. Siamese and oriental breed cats had a high incidence of mediastinal lymphoma, suggesting a genetic predisposition.[46]

PATHOLOGY AND NATURAL BEHAVIOR

Epithelial, mesenchymal, neuroendocrine, and discrete/round cell neoplasia can all be found in the intestinal tract. In cats and dogs, lymphoma is the most common type of small intestinal neoplasia, followed by adenocarcinoma. Subtypes of feline intestinal lymphoma include lymphocytic, lymphoblastic, epitheliotropic and large granular lymphocyte types. Gastrointestinal
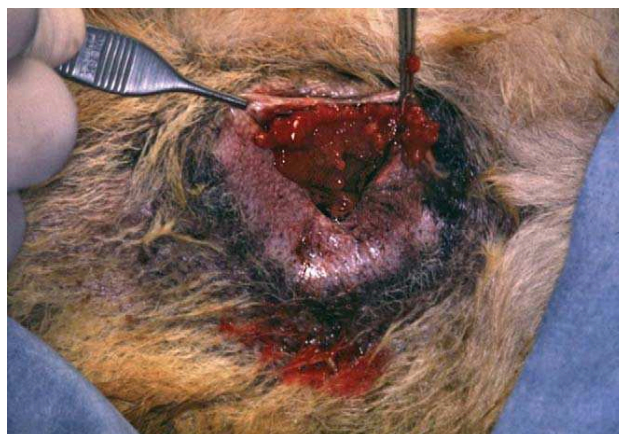
Figure 21-20

Cobblestone appearance to a rectal adenocarcinoma. Dogs with this tumor type live an average of 12 months following surgical excision.[48] *Courtesy of Dr. Eric Pope.*

stromal tumors (GISTs) have also been reported in dogs, a tumor type previously described as leiomyosarcoma and known to be associated with mutated tyrosine kinase receptor activity in humans. Other tumors include leiomyosarcoma in both dogs and cats, carcinoids in dogs, and mast cell tumors in cats. Extramedullary plasmacytoma, extraskeletal osteosarcoma, and hemangiosarcoma have also been rarely reported. While most small intestinal neoplasia is malignant in dogs, most rectal tumors are benign polyps, adenomas, or carcinoma *in situ*[28,47] (Figure 21-20).

Most alimentary *adenocarcinoma* is found in the small intestine.[1,29,36] However, the colon and rectum are a more common site in dogs.[4,5] Of colorectal adenocarcinomas, the rectum is a more common site than the colon.[48] The cecum is more likely to develop leiomyosarcoma or GIST than adenocarcinoma.[5,22] Histologic descriptors for *carcinoma* of the intestine include adeno- (forming glands), mucinous (>50% mucin), signet ring (>50% cells have intracellular mucin), and undifferentiated or solid (no evidence of gland formation).[4] Grossly, colorectal adenocarcinomas may demonstrate a pedunculated (especially in the distal rectum), cobblestone (middle rectum), or annular (middle rectum) appearance, which may relate to behavior and prognosis[5,48,49] (Figure 21-21).

Adenomatous *polyps* are found in the rectum of dogs and carcinoma *in situ* in both the colon and rectum. Most lesions are solitary, although multiple and diffuse lesions can be seen and result in increased local recurrence.[28] In cats, polyps are more common in the duodenum.

The term *carcinoid* refers to tumors that arise from the diffuse endocrine system rather than the intestinal epithelium, despite great histologic similarity to carcinomas. Carcinoid cells contain secretory granules, which may

A   B
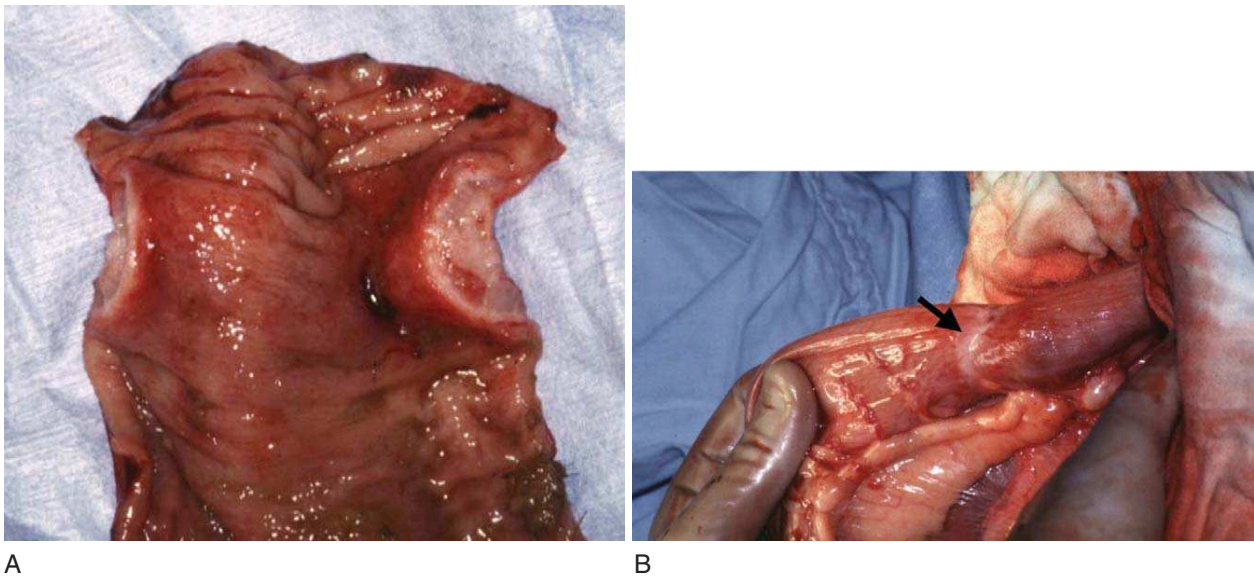
Figure 21-21

An annular form of colonic adenocarcinoma causing a stricture. The thick band of tissue **(B)** creating the stricture is seen on cross section **(A)**. In one study, dogs with this type of tumor survived an average of only 1.6 months.[48] *Courtesy of Dr. Eric Pope.*

contain substances such as 5-hydroxytryptamine (serotonin), secretin, somatostatin, and gastrin, among others.[4] Immunohistochemistry for cytokeratin and for secretory substances such as serotonin may be positive.[50] Described in many species, carcinoids may occur in both the large and small intestine and frequently metastasize to the liver.[2,5,50] Carcinoids may follow an aggressive and debilitating clinical course.[50]

GISTs are well documented in people and have been reported in dogs. These nonlymphoid tumors of mesenchymal origin are predominantly a type of leiomyosarcomas and some but not all leiomyomas. They are distinguished by high vimentin immunoreactivity, low alpha smooth muscle actin reactivity, CD117 (KIT) reactivity in approximately half the tumors, and a site predilection for the large intestine.[30,51] Approximately 30% of GISTs will metastasize. Mutations were identified in KIT exon 11 encoding the juxtamembrane domain in two of four cases examined.[30] One study proposed that positive CD117 staining for c-kit should distinguish GISTs from leiomyosarcomas. When stratified as such, only 2 of 28 cases of GIST metastasized (7%). These investigators also found that GIST was significantly more likely to occur in the large intestine, specifically the cecum, and leiomyosarcoma in the small intestine.[52]

Intestinal *lymphoma in dogs* occurs in the stomach and small intestine equally and more often in both than the large intestine. Lesions are typically diffuse and neoplastic cells infiltrate the submucosa and lamina propria. Additional visceral and systemic involvement may be seen.

Intestinal *lymphoma in cats* was originally thought to be predominantly of B cell origin, resulting from its origin in Peyer's patches and germinal centers. However, evidence suggests that incidence of T cell lymphoma may equal or exceed that of B cell.[19,33,40] There is no clear association between the presence of FeLV antigens in tissue and clonality (B versus T cell).[40] Within a diagnosis of intestinal lymphoma, subtype also impacts behavior. In one series, cats with lymphocytic/small cell lymphoma experienced a 69% complete remission rate with prednisone and chlorambucil for a median survival time of nearly 2 years, whereas cats with lymphoblastic lymphoma had only an 18% complete remission rate with combination chemotherapy for a median survival time of less than 3 months. Cats with lymphoblastic lymphoma were more likely to have a palpable abdominal mass and require surgery for intestinal obstruction than cats with lymphocytic lymphoma.[34]

Other unique subsets of feline intestinal lymphoma include epitheliotropic and large granular lymphocyte lymphoma. Most of these cats are serologically negative for FeLV. In a series of 10 cases, *feline epitheliotropic lymphoma* was determined immunohistochemically to be T-cell in origin. Although great overlap of values occurred, there was a significantly greater percentage of intraepithelial lymphocytes in neoplastic compared to normal cats and cats with inflammatory bowel disease.

The percentage of these cells did not differentiate between epitheliotropic and nonepitheliotropic lymphoma. Most (80%) of affected lymphocytes were small. As with epitheliotropic cutaneous lymphoma, microabscesses were often seen. Intraepithelial lymphocytes are richer in villous than crypt epithelium, suggesting that this diagnosis may be reliably made with endoscopic biopsies. This disease may represent a continuum from inflammatory bowel disease.[33]

By contrast, *large granular lymphocyte lymphoma* (also called *globule leukocyte* and *granulated round cell tumor*) often has a rapidly progressive and fatal course. Few attempts at therapy have been reported in the literature, although one cat did well for over a year following surgical resection until the tumor recurred.[53] These tumors are distinguished by heterogenous cytoplasmic granules (azurophilic on cytology and eosinophilic on histopathology with routine hematoxylin and eosin staining) and are commonly seen in the intestinal tract (especially jejunum), occasionally with leukemic cells.[54,55] Perforin-like immunoreactivity has been demonstrated and may help distinguish these from other lymphomas.[13] Although large granular lymphocytosis has been reported in dogs, solitary intestinal lymphoma of this type has not.

Extramedullary plasmacytoma (EMP) refers to solitary tumors with no evidence of systemic multiple myeloma. Rare reports of gastrointestinal EMP in dogs and cats exist. In one series, one fourth of EMPs were found in the digestive system, most in the mouth.[56] One case report in a dog with EMP of the colon and rectum was associated with monoclonal gammopathy.[57] Another tumor is *extraskeletal osteosarcoma*, which has been reported in the duodenum of a cat. This cat had no evidence of metastasis at diagnosis but clinical signs recurred and the cat died 4 months following excision.[58] Three of 55 extraskeletal osteosarcoma of cats were of intestinal origin.[59] Intestinal *mast cell tumors* are cited as the third most common tumor following lymphoma and adenocarcinoma in cats, but incidence and behavior are poorly reported. They have been confused with carcinoids but are distinct.[11] They may present as an eosinophilic enteritis, and conversely eosinophilic enteritis may mimic intestinal tumors.[60,61] Finally, one dog was diagnosed with *ganglioneuroma* of the rectum and had long-term survival following surgical resection.[62]

When intestinal tumors metastasize, sites of predilection in decreasing frequency include mesenteric lymph nodes (especially adenocarcinoma), liver (especially leiomyosarcoma), mesentery, omentum, spleen, kidney, bone, peritoneum/carcinomatosis, lung, testes, and skin.[10,31,36,49,63,64] A monkey with intestinal adenocarcinoma had metastasis to the pancreas and lymph nodes.[65] Lymphoma is often a systemic disease and 25% of dogs and 80% of cats with gastrointestinal lymphoma will have concurrent involvement of other organs.[12,21]

Molecular Aspects

With an increasing armamentarium of molecular diagnostics, insights into the pathogenesis, progression, and prognosis of tumors are constantly emerging. Cellular adhesion and invasion, stromal remodeling, and alterations in tumor suppressor genes all may play a role in the development and progression of intestinal neoplasia.

Tenascin-C is a glycoprotein of the extracellular matrix that likely is produced by intestinal stromal cells under the influence of adjacent epithelial cells.[47] Its primary role is to mediate cell anti-adhesion.[66] Myofibroblasts may be important in tissue remodeling in which tenascin may play a role. The pattern of tenascin expression may be different in benign versus malignant intestinal epithelial tumors.[47] Similarly, the extracellular matrix components *versican* and *hyaluronan* play a role in cell anti-adhesion and motility and have been evaluated in canine intestinal adenomas and adenocarcinomas. Versican, a large chondroitin sulphate proteoglycan, may bind hyaluronan. While weak versican staining is present in submucosal stroma of normal tissue, and none in stroma of adenomas, there is strong expression in adenocarcinoma stromal tissue that correlated well with grade and depth of invasion. Hyaluronan, by contrast, was highly expressed in both adenomas and adenocarcinomas and did not correlate to grade and depth of invasion.[67]

Other adhesion molecules, including the interconnected β-catenin and E-cadherin, have been investigated in canine colorectal tumors. The loss of either can lead to loss of intercellular adhesiveness. All colorectal adenomas examined in one study and almost half the carcinomas stained positively for β-catenin, suggesting a role in carcinogenesis as has been shown in human colorectal adenomatous polyposis coli.[68]

The expression of the tumor suppressor gene p53 has been evaluated in canine intestinal tumors and a low incidence of positive expression (23%) was found in gastrointestinal adenocarcinomas,[69] while nearly half the benign and malignant colorectal epithelial tumors examined expressed p53 as determined by immunohistochemistry (IHC).[32] Another study found 23% of polyps and 17% of carcinomas in the colon and rectum to express p53, and 15% of carcinomas in the small intestine positive.[68] Genetic analysis of a cecal anaplastic carcinoma in a dog revealed a mutation in codon 249 for p53, a hot spot in human tumors.[70] Although p53 staining does not seem to differentiate between benign and malignant tumors, its presence suggests a role for p53 in some canine intestinal tumors.

Measures of cellular proliferation include markers such as argyrophilic nucleolar organizer regions (AgNORs), which will stain with silver. In feline intestinal lymphoma, AgNORs did not correlate with remission rate or duration, or with survival time.[18]

Cyclooxygenase (COX) enzymes are responsible for prostaglandin synthesis and COX-2 is overexpressed in many head/neck and genitourinary tumors, creating a possible therapeutic target. COX-2 has been identified in both benign and malignant small intestinal and colorectal epithelial tumors in dogs, but one study found no COX-2 staining in 13 intestinal tumors in cats.[71,72]

HISTORY AND CLINICAL SIGNS

The duration of clinical signs prior to presentation averages 6 to 8 weeks but can range from less than 1 day to several months.[10,21,22] Clinical signs include weight loss, diarrhea, vomiting, and anorexia, and less frequently melena, anemia, and hypoglycemia (with smooth muscle tumors).[2,10,14,24,26,33,34,37] Clinical signs often relate to location of the tumor within the gastrointestinal tract. Higher lesions more commonly result in vomiting, small intestinal lesions in weight loss, and large bowel lesions in hematochezia and tenesmus.[31,29] Although carcinoids may secrete endocrine substances, clinical signs rarely reflect hypersecretion.[4] Dogs and cats may present as a result of intestinal obstruction and although uncommon, perforation and peritonitis can occur.[33] Although smooth muscle tumors are located within the muscular layer of the intestines, and not within the lumen, anemia and melena have been reported.[22,23]

Paraneoplastic Syndromes

Rare cases of paraneoplastic cutaneous, hematologic and hyperviscosity syndromes have been reported in dogs and cats with intestinal tumors.[73,77] One dog with neutrophilic leukocytosis, monocytosis, and eosinophilia experienced complete resolution of hematologic abnormalities following excision of an adenomatous rectal polyp.[74] Hypereosinophilic syndrome has been reported in a cat with intestinal T cell lymphoma, the suggested cause was interleukin-5 secretion by the neoplastic lymphocytes.[75] Erythrocytosis managed with periodic phlebotomy was related to a cecal leiomyosarcoma in a 14-year-old dog. The diagnosis was made at postmortem 2 years later, and erythropoietin mRNA and protein were isolated from tumor cells, suggesting ectopic erythropoietin production as the cause of the erythrocytosis.[76]

DIAGNOSIS

Physical Examination

An abdominal mass may be palpated upon initial examination in approximately 20% to 40% of dogs with lymphoma[21,26] and 20% to 50% of dogs with nonlymphomatous solid intestinal tumors.[10,29,31] Other physical findings include pain and fever in 20% of dogs with lymphoma.[21] Digital rectal examination may identify masses or annular strictures due to rectal tumors or polyps in as high as 63% of dogs.[29,48]

Abdominal masses are often readily palpated in cats with both lymphoma and adenocarcinoma. Approximately 50% of cats with nonlymphomatous tumors will present with a palpable mass.[14,31] Abnormal abdominal palpation is common in cats with lymphoma as 86% have a palpable mass.[15,33] Dehydration is also common.[14,31]

Clinical Pathology

CBC

Anemia is common in dogs and cats with intestinal tumors, which may occur in conjunction with melena and elevated blood urea nitrogen (BUN).[10,22,23,29,31,33,15] Leukogram changes include leukocytosis in 25% to 70% of dogs and 40% of cats.[10,14,23,31] Left shift may be seen as well as monocytosis in some patients.[31,33]

Chemistry Profile

Biochemical abnormalities are similar between dogs and cats with intestinal tumors. Hypoproteinemia may be present in one fourth to one third of patients.[10,14,15,23,26,29] Other abnormalities include elevated liver enzymes, specifically alkaline phosphatase in 15% to 33% of dogs and up to 85% of cats with nonlymphomatous neoplasia.[10,23,29,31,33] In one series, a high cholesterol was seen in 41% of cats with nonlymphomatous tumors.[31] An elevated BUN has been reported in 13% of dogs and 30% of cats with intestinal adenocarcinoma.[10,14] This may result from concurrent renal insufficiency, absorption following intestinal bleeding, or dehydration. While some cats present with hyperglycemia,[31] smooth muscle tumors can cause hypoglycemia in up to half of patients as a result of insulin-like growth factor secretion.[22] Dogs may also have increased amylase and electrolyte disturbances,[29] and patients with lymphoma may be hypercalcemic.[15] Serum alpha 1-acid glycoprotein (AGP), an acute phase reactant protein, may be increased in cats with cancer, but lacks specificity and clinical utility.[78,79]

Imaging

Plain and contrast abdominal radiographs

In dogs and cats with intestinal lymphoma, concurrent enlargement of liver, spleen, or mesenteric lymph nodes may be seen.[21] Plain abdominal radiographs may reveal an abdominal mass in approximately 40% of both dogs and cats, though some reports are higher for

Figure 21-22

The arrow indicates an obstructing tumor on contrast radiography. The thin trail of barium is all that will pass through the lumen of the tumor and the obstruction is evidenced by the dilated segment of small bowel adjacent and oral to the tumor. *Courtesy of Dr. Jimmy Lattimer.*

solid tumors and lower for lymphoma.[10,14,15,21,23,31] Intestinal lymphoma may be more difficult to identify on plain radiography because of other organ involvement, peritoneal effusion, or diffuse intestinal lesions. An obstructive pattern may also be seen on plain radiographs with incidence ranging from 10 to 75%.[10,23,29,31] Other abnormalities may include poor serosal detail and thickening of the stomach wall.[15]

Contrast radiography, though used less in the face of advances in ultrasound, has often been used to evaluate patients with signs of primary gastrointestinal disease. Contrast radiography can help document an obstruction, localize a tumor, and view areas of the gastrointestinal tract that are difficult to image with ultrasonography because of gas accumulation (Figure 21-22). Contrast radiographs may reveal filling defects in approximately half the cats and dogs with gastrointestinal neoplasia.[31] In dogs with gastrointestinal lymphoma, all 12 dogs examined had abnormal contrast series.[21] Eighty-seven percent of cats with intestinal adenocarcinoma in one series showed evidence of partial or complete obstruction.[14]

Thoracic radiographs
Thoracic radiographs are critical to the complete evaluation of the cancer patient; however, yield is low with few patients presenting with pulmonary metastasis.[10,14,22,23,29,31] For cats and dogs with lymphoma, enlarged sternal or perihilar lymph nodes, pleural effusion, or diffuse interstitial changes may be seen.[15,21]

Abdominal ultrasound
This imaging modality can localize the tumor, evaluate other sites of metastasis/involvement, improve staging, guide needle aspiration, needle biopsy, and assist in treatment planning. Ultrasound is a more sensitive diagnostic test than radiographs for identifying a mass.[10,22,27,80] Ultrasound is also less time consuming than contrast radiography.

Ultrasound findings in dogs and cats with intestinal neoplasia most consistently include bowel wall thickening and loss of normal wall layering.[29,80,81] Intestinal lymphoma in dogs often results in long segments of involved bowel, and either a solitary mass or diffusely thickened bowel loops in cats.[34,81] Adenocarcinoma in cats has been described as having mixed echogenicity and was asymmetric in 3 of 5 cats.[80] In one study, two thirds of dogs with intestinal adenocarcinoma had hypoechoic tumors, and most had decreased motility. Masses averaged 4 cm long with a median wall thickness of 1.2 cm.[5,29] Smooth muscle tumors are characteristically large (median diameter 4.8 cm), anechoic, or hypoechoic, and a muscular layer origin may be identified. Leiomyomas may have a smooth contour.[27]

Ultrasound, while not diagnostic alone, has also proven useful in differentiating neoplastic from non-neoplastic intestinal disease. Dogs with tumors have significantly thicker intestinal walls, and 99% have a loss of wall layering as compared to a maintenance of wall layering in 88% of dogs with non-neoplastic disease (Figure 21-23). Dogs with a loss of wall layering are more than 50 times more likely to have a tumor than enteritis. Additionally, dogs with walls thicker than 1 cm are nearly four times as likely to have a tumor, and those with focal lesions are nearly 20 times as likely.[81]

In a series of 14 cats with carcinomatosis, three of which were a result of small intestinal tumors, the hallmark ultrasonographic finding (100% of cats) was the presence of masses in the double sheet portion of peritoneum that connects the visceral and parietal portions. All cats also had free peritoneal fluid.[35]
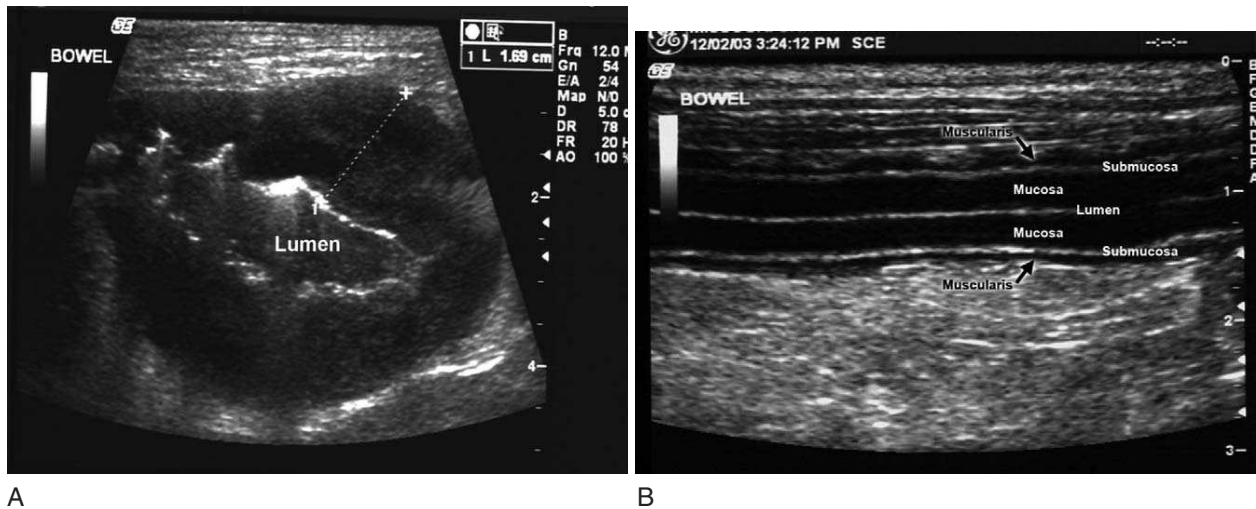
Figure 21-23

A cross-sectional ultrasound image of a segment of small intestine with lymphoma **(A)** is compared to a longitudinal view of a segment of normal small intestine **(B)**. Note that the clearly defined intestinal layers in the normal tissue are completely effaced in the tumor tissue. A loss of layering is strongly supportive of neoplasia. The diseased bowel is also markedly thickened, suggesting neoplasia. *Courtesy of Dr. Stephanie Essman.*

Endoscopy and laparoscopy

Endoscopic findings in dogs with intestinal lymphoma include an irregular cobblestone or patchy erythematous appearance to the duodenal mucosa and poor distensibility and elasticity of the duodenal wall.[26] There may be significant interobserver variation in the interpretation of biopsy samples. In one study, blinded pathologists assigned a degree of mucosal cellular infiltrate as severe as neoplasia in five clinically normal research dogs.[82] Interobserver variations are likely to be more pronounced with small tissue samples, and this is a limitation of these less invasive approaches.

Exploratory laparotomy

When non- or minimally invasive diagnostics fail to confirm a diagnosis, an exploratory laparotomy may be indicated for a dog or cat with persistent signs of gastrointestinal disease. Benefits include direct visualization of all abdominal viscera, the ability to collect full thickness biopsies of all segments of intestine and other viscera, and the potential to allow a therapeutic surgical intervention such as resection and anastamosis. In a series of dogs with gastrointestinal lymphoma, endoscopic biopsies were sometimes difficult to interpret because of lymphoplasmacytic infiltrate; however, biopsies obtained by laparotomy confirmed the diagnosis in all cases undergoing surgery.[21] Carcinomatosis can also be evaluated at surgery (Figure 21-24).[14]

THERAPY AND PROGNOSIS

Surgery

With the exception of lymphoma, surgical resection is the primary treatment for intestinal tumors. So long as extensive extraserosal invasion or adhesions do not complicate the surgical approach, complete excision is often possible. For dogs and cats without evidence of local or distant metastasis, long-term survival is possible, though some patients may go on to develop metastasis. The overall 1-year survival rate is approximately 40% for dogs with solid small intestinal tumors.[10] For cats with adenocarcinoma, approximately 50% will metastasize to the local lymph nodes, 30% to the peritoneal cavity (carcinomatosis), and 20% or less to the lungs.[2,31,36] Dogs have similar rates of metastasis to lymph nodes for both adenocarcinoma and leiomyosarcoma, though liver is usually the second most frequent site.[5,10,31] Perioperative mortality can approach 30% to 50% as a result of sepsis, peritonitis, or owner decision for euthanasia when nonresectable tumors are present.[10,22]

Regarding surgical techniques for resection and anastamosis of small intestine, stapling techniques have been shown to be equivalent to hand suturing.[83] For dogs with colorectal adenocarcinoma, local excision yielded a median survival of 22 months compared to 15 months for stool softeners alone; polyps also fare well with median survival times of 2 years or more
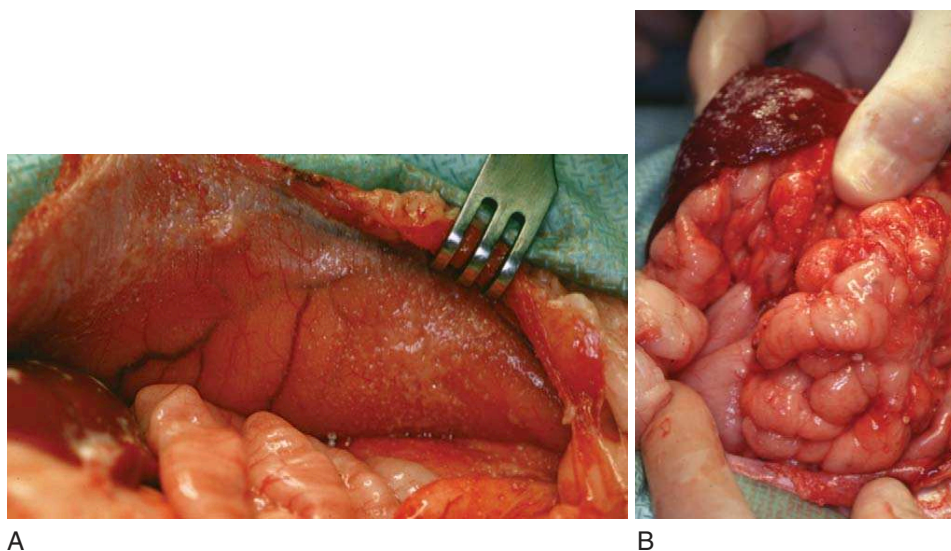
Figure 21-24

Carcinomatosis discovered at exploratory laparotomy. Note the irregular peritoneal surface instead of a normal glossy appearance **(A)** and the multiple serosal implants **(B)**. *Courtesy of Dr. F. A. Mann.*

following polypectomy.[48] In contrast, dogs with small intestinal adenocarcinoma are associated have shorter mean survivals of 12 days without treatment, and 114 days with surgical resection. Others report 7- and 10-month survivals following surgery.[10,29,31] Dogs with leiomyosarcoma who survive the perioperative period fare better with median survival times of 1.1 and 2 years reported.[22,23] In one series, the median postoperative survival for 28 dogs with GIST was approximately 3 years (1 year if postoperative deaths were included) versus 233 days for 10 dogs with leiomyosarcoma, though the difference was not statistically significant.[52]

Transrectal endoscopic removal of benign canine rectal tumors that would otherwise require rectal pull-through surgery or pubic osteotomy afforded five of six dogs significant improvement in quality of life, with three dogs cured.[84] Standard treatment with surgery yields a 41% recurrence of clinical signs, and 18% of dogs experienced transition to malignancy with tumor recurrence.[28] Surgical removal of duodenal polyps in cats typically affords a cure.[16]

In cats with small intestinal adenocarcinoma, there is significant perioperative risk; however, cats who live 2 weeks beyond surgery may experience long-term control (Figure 21-25). In two series, cats that did not have their tumors resected were euthanized or died within 2 weeks of surgery.[14,31] Mortality rate is high in the first 2 weeks following surgery.[36] For cats that survived 2 weeks beyond surgery, mean survival was 15 months in one report and only 20 weeks (median) in another.[14,37] Cats with carcinomatosis may receive palliation from resection of the primary mass. Two cats lived 4.5 and 28 months following surgery.[14]
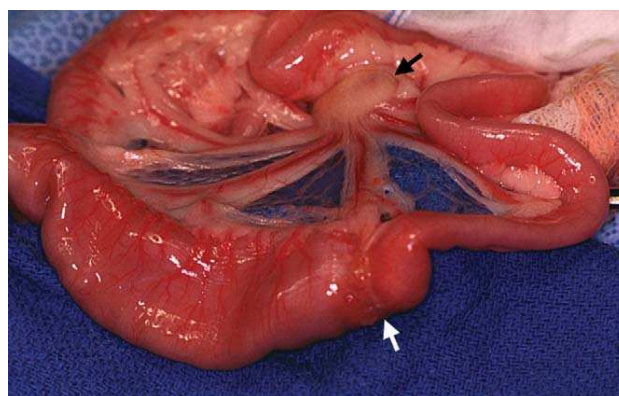
Figure 21-25

Intestinal obstruction as a result of adenocarcinoma (*white arrow*). Note the distension of the jejunum oral to the mass as compared to the normal diameter aboral to the mass. There is also an enlarged lymph node (*black arrow*). *Courtesy of Dr. Eric Pope.*

In cats with large intestinal neoplasia, survival following surgery alone was approximately 3.5 months for lymphoma, 4.5 months for adenocarcinoma, and 6.5 months for mast cell tumor. Adjuvant chemotherapy significantly improved survival for cats with adenocarcinoma, but not for cats with lymphoma.[17]

Lymphoma is treated primarily with chemotherapy, except when intestinal perforation, intestinal obstruction, or the need for a biopsy necessitates surgery (Figure 21-26). Surgery and chemotherapy did not improve survival compared to chemotherapy alone for cats with alimentary lymphoma.[19]

A

B

C
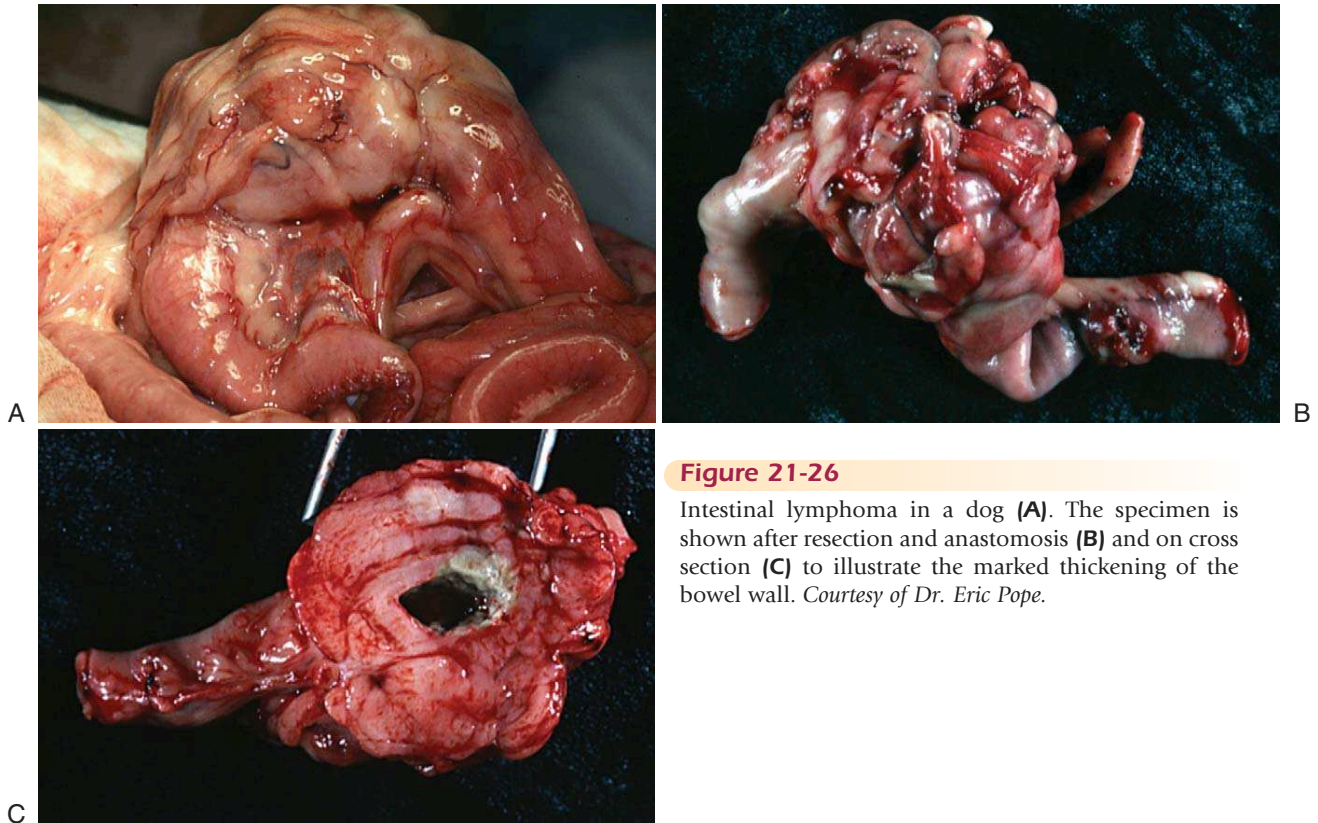
Figure 21-26

Intestinal lymphoma in a dog **(A)**. The specimen is shown after resection and anastomosis **(B)** and on cross section **(C)** to illustrate the marked thickening of the bowel wall. *Courtesy of Dr. Eric Pope.*

Chemotherapy

No randomized studies exist to confirm or deny benefit of adjuvant chemotherapy following resection of intestinal tumors in people, dogs, and cats. One retrospective study in cats with colonic adenocarcinoma did show a significant survival advantage for cats receiving adjuvant doxorubicin, with a median survival of 280 days with and 56 days without chemotherapy.[17] For carcinomatosis, intracavitary therapy may be helpful with carboplatin for cats or cisplatin or 5-fluorouracil for dogs.[85a] Two dogs with leiomyosarcoma received adjuvant doxorubicin chemotherapy following surgical resection. One had metastasis at surgery and died 4 months later, and the other was lost at over 2 years; however, several other dogs in that series were long-lived that did not receive chemotherapy.[22] Two dogs with adenocarcinoma also received chemotherapy following surgery, and both survived longer than 17 months.[29]

In dogs with gastrointestinal lymphoma, of eight dogs treated in one report, all were euthanized by 14 weeks.[21] In cats with intestinal lymphoma, median survival time is typically 6 to 9 months, though many studies comment that a subset of cats do well, living longer than 2 years.[15,24,34] This may relate to histologic subtype as cats with lymphocytic lymphoma (22.8 months median survival) fared vastly better than those with lymphoblastic lymphoma (2.7 months).[34] General treatment of feline lymphoma is discussed elsewhere (Chapter 31, Section B), but treatment regimens reported typically include vincristine, doxorubicin, prednisone, and cyclophosphamide, and chlorambucil and prednisone for cats with lymphocytic lymphoma.[34]

In dogs with rectal tubulopapillary polyps, piroxicam (oral or suppository) has been reported to result in considerable improvement in clinical signs and may represent a noninvasive treatment option.[85b] The use of cyclooxygenase inhibitors as preventative following surgical intervention for colonic tumors has been suggested and warrants further investigations. Additionally, preliminary data of a retrospective study found a significantly reduced incidence of cancer in dogs with a history of NSAID use.[93]

Radiation Therapy

Because of concern for toxicity to surrounding abdominal viscera and the inability to reliably irradiate the same intestinal tissue region each day because of intestinal mobility, radiation therapy is seldom used in the treatment of intestinal tumors.

PROGNOSTIC FACTORS

Intestinal perforation does not appear to be a negative prognostic factor for leiomyosarcoma as dogs surviving the perioperative period enjoyed prolonged survival in one series.[22] For colorectal tumors, treatment is prognostic with local excision significantly better than palliative care. Gross appearance, though not statistically examined, may determine outcome as dogs with annular, obstructing masses survived a mean of 1.6 months; nodular or cobblestone masses, 12 months; and single, pedunculated masses, 32 months.[48]

For nonlymphomatous small intestinal tumors in dogs, metastasis at the time of surgery resulted in significantly shorter survival times (3 versus 15 months). One-year survival was 20% for dogs with lymph node involvement, as compared to 67% without.[10] In another study, however, dogs with visceral metastasis from leiomyosarcoma enjoyed prolonged survival following resection of the primary tumor (21.7 months).[22] In one study, males fared significantly better than females for small intestinal adenocarcinoma, though numbers were small.[29]

The strongest prognostic factor for cats with intestinal lymphoma is response to treatment. Cats achieving a complete resolution of clinical signs typically fare significantly better than those that do not.[19,33,86] For 103 cat with lymphoma, 28 of which were intestinal, negative prognostic factors were FeLV positivity (median survival, 3 months if positive, 17 months if negative for early stage disease; not prognostic for advanced stages) and advanced stage of disease.[25] FeLV status did not affect outcome in other studies.[19,24] Immunophenotype does not appear to be a prognostic factor in either dogs or cats for intestinal lymphoma specifically.[19] For large intestinal lymphoma, cats with surgery fared equally poorly with and without adjuvant chemotherapy (median survival of just over 3 months in both groups). Cats with adenocarcinoma, however, survived significantly longer if they received subtotal colectomy (138 days versus 68 days with mass excision), postoperative doxorubicin (280 days with versus 56 days without), and had negative lymph nodes at surgery (259 days negative versus 49 days positive).[17]

COMPARATIVE ASPECTS

While cancer of the large intestine and rectum is well characterized in people, small intestinal neoplasia is rare. This is in contrast to veterinary-based medicine where malignant neoplasia is more common in the small intestine. This may reflect differences in physiology, diet, or genetics among species. The difference in species may also be a matter of proportions in that people develop colorectal cancers more commonly. As in animals, tumors of the small intestine of people are usually malignant. Intake of red meat, salt-cured foods, and fat are associated with an increased risk of small intestinal neoplasia, though tobacco and alcohol use are not.[87] Diagnostic evaluation is similar to that described in animals, although advanced imaging such as computed tomography is more often used. Most diagnoses are made at surgery, and 5-year survival rates average just over 20%.[87]

In contrast to the rarity of small bowel neoplasia, large bowel/colorectal cancer is one of the most frequently diagnosed cancers in both men and women. Risk factors include genetic predisposition/familial history, tobacco and alcohol intake, advanced age, and predisposing medical conditions, among others. Colorectal cancer development may further be influenced by intake of red meat (especially fried), a low-fiber or high-fat diet, obesity, fecal pH, and fecal mutagens. Among genetic risk factors, polymorphism in colonic enzymes and mutations leading to familial adenomatous syndromes are uncommon but are important as models of carcinogenesis. In most familial polyposis syndromes, the adenomatous polyposis coli (APC) gene is mutated to create a premature stop codon, thus encoding a truncated, nonfunctional protein. The multistage progression from benign polyp to carcinoma is well understood and underscores the importance of early detection.[88] In contrast, hereditary nonpolyposis colon cancer (HNPCC) develops without known premalignant polyps. It is inherited via autosomal dominance with high penetration and is characterized by microsatellite instability.[89]

Severe celiac disease in people is associated with an increased risk of lymphoma. Progression to lymphoma from inflammatory bowel disease (IBD), especially in cats, has been postulated but not confirmed. Two of 97 cats with lymphoma had a history of IBD in a population of cats examined for comparison to a group of cats with IBD at one institution during the same time period.[90]

The most clinically important aspects of comparative oncology when considering intestinal neoplasia in people are the use of cyclooxygenase inhibitors in treatment and prevention of colorectal neoplasia and the use of tyrosine kinase inhibitors (TKI). In people, KIT mutations in gastrointestinal stromal tumors have led to the use of imatinib mesylate, a TKI that inhibits KIT.[91] As KIT has been shown to be mutated in some canine gastrointestinal stromal tumors, TKIs may benefit this population as well.

Cyclooxygenase inhibition by nonsteroidal anti-inflammatory drugs (NSAIDs) will decrease the incidence of colorectal cancer and decrease mortality by 40% to 50%.[92] Among the proposed mechanisms of action, prostaglandin production is thought to be related to tumor progression and therefore inhibition leads to cancer prevention. Additionally, noncyclooxygenase pathways include inhibition of transcription

factors and induction of nuclear hormone receptors that lead to cellular differentiation.[92]

Therapy in people is similar to that in companion animals. Surgical resection is the primary mode of therapy, with adjuvant targeted or traditional chemotherapy in many cases, especially if patients present with lymph node metastasis or unresectable disease. Transanal endoscopic resection is used when possible for rectal tumors, and this technique has been performed with some success in dogs. Surgery alone is often inadequate with up to 90% of GISTs relapsing after surgical excision alone and lymph node metastasis in small intestinal adenocarcinoma in up to 70% of patients.[87] GISTs are reportedly radioresistant, but TKIs may improve prognosis for unresectable and metastatic GISTs. Risk of metastasis in GISTs is related to tumor size and mitotic rate.[91] Adjuvant chemotherapy is used in colon cancer, with 5-fluorouracil based combinations providing the best control, and one study demonstrates still greater benefit when combined with a platinum agent. There is no known benefit for adjuvant chemotherapy with small intestinal neoplasia, though a paucity of studies exist.[94] Radiotherapy is used primarily for areas of the gastrointestinal tract that are not very mobile, such as the stomach and rectum.

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SECTION H
Perianal Tumors

Michelle M. Turek and Stephen J. Withrow

The perianal area of dogs contains several glands and structures from which tumors may develop. Perianal, or circumanal, glands are located in the dermis in a circular fashion around the anus and are also scattered in areas on the prepuce, tail, pelvic limbs, and trunk.[1] These are commonly referred to as "hepatoid glands," owing to their morphologic resemblance to hepatocytes, and are considered nonsecretory, sebaceous glands in the adult dog.[1-3] The anal sacs represent blind cutaneous diverticula that are located on each side of the anus at the 4 o'clock, and 8 o'clock positions. Located in the connective tissue surrounding these diverticula are distinct apocrine sweat glands that empty their secretions into the lumen of the anal sacs. The most frequently observed tumors of this region in dogs include perianal sebaceous adenoma, perianal sebaceous adenocarcinoma, and apocrine gland adenocarcinoma of the anal sac (Table 21-9).

INCIDENCE AND RISK FACTORS

Perianal adenomas (circumanal, hepatoid tumors) represent the majority, 58% to 96%, of canine perianal tumors.[1,4] Development of these benign tumors appears to be sex hormone-dependent, where growth is either stimulated by androgenic hormones or depressed by estrogenic hormones.[1,5,6] The older, intact male is at high risk.[1,4,5] The mean reported age is 10 years.[4] A high incidence of associated testicular interstitial cell tumors has been reported for males with adenomas, supporting testosterone production as a cause.[5] However, a true cause-and-effect relationship has not been clarified since interstitial cell tumors are a common incidental finding in non-tumor-bearing, older intact males. Perianal adenomas in the female occur almost exclusively in ovariohysterectomized animals in which low levels of estrogen do not suppress tumor growth. Rarely, testosterone secretion from the adrenal glands, occasionally accompanied by signs of hyperadrenocorticism, may stimulate perianal adenoma formation in the female.[7a,7b] Cocker spaniels, beagles, bulldogs, and Samoyeds may be predisposed.[1,5]

TABLE 21–9 Perianal Tumors

	MALE		FEMALE	
	Benign	Malignant	Benign	Malignant (May Occur in Males)
Cell type	Sebaceous	Sebaceous (rare apocrine)	Sebaceous	Apocrine (anal sac)
Tumor type	Perianal adenoma	Perianal adenocarcinoma	Perianal adenoma	Anal sac adenocarcinoma
Frequency	Common	Rare	Rare	Rare
Hormonal factors	Usually intact, testosterone dependent (?)	None	Ovariohysterectomized (i.e., lack of estrogen)[a]	Often ovariohysterectomized but no proven hormone regulation
Location and appearance	Superficial hairless perineum; single, multiple, or diffuse; may be on prepuce or tailhead	Usually single, invasive, often ulcerated	Superficial and single	Subcutaneous at four or eight o'clock, firm and fixed; primary tumor may be very small with metastasis
Paraneoplastic syndromes	None	None (very rarely hypercalcemia)	None	50%–90% have hypercalcemia
Metastatic pattern	None	First to regional nodes, then further, up to 50% of time, especially with multiple recurrence	None	Very common to regional lymph nodes and then further
Special workup	None; cytology may have difficulty telling benign from malignant	Caudal abdominal X rays or ultrasound	None	Caudal abdominal X rays or ultrasound possibly chest X rays; calcium levels and renal function
Treatment	Castration, surgical or cryosurgical removal of tumors[b]	Wide excision and lymph-adenectomy if involved; radiation or chemotherapy if inoperable; castration of little benefit	Surgery or cryosurgery[b]	Wide excision of primary, lymphadenectomy, consider radiation postop to primary and metastatic lymph node sites
Prognosis	Excellent, less than 10% recurrence rate	Fair to good (tumors <5 cm do well); recurrence is common but may take many months and several surgeries can be done	Excellent	Poor to fair; less than 40% 1-year survival

[a]If multiple or large (malelike), consider testosterone secretion from adrenal (with or without Cushing's signs).
[b]Estrogens will cause regression but carry risk of bone marrow suppression. Adenomas will respond to radiation therapy, but surgery is cheaper, faster, and safer.

Perianal adenocarcinoma, a malignant tumor of the perianal glands, occurs much less frequently than its benign counterpart, representing only 3% to 21% of all tumors in this region.[1,4] Average age of affected dogs is 11 years.[4,8] Tumors occur in castrated or intact males, as well as in females, implying no hormonal dependency.[5,8] Large-breed males appear to be overrepresented.[8]

Apocrine gland adenocarcinoma of the anal sacs occurs at relatively low frequency in the dog, representing 17% of perianal tumors and 2% of all "skin" tumors.[4,9] It is the most common malignancy in this area in older female dogs. Earlier reports have suggested a female predilection;[4,10-12] however, approximately equal sex distribution was found in two larger studies.[13,14] The average age of dogs diagnosed with this disease is 10 to 11 years.[4,10,11,13,14] Dogs as young as 5 years have been reported,[10,11,14,15] suggesting that evaluation of the perineum and palpation of the anal sacs should be a routine part of the physical examination in every adult dog.[14] Hormonal dependence has not been suggested. Benign tumors of the anal sac are very rare.

Because cats do not have glands analogous to the perianal sebaceous glands in the dog, perianal adenoma and perianal adenocarcinoma are uncommonly recognized in this species. Apocrine gland adenocarcinoma of the anal sac is also rare in the cat.[16,17]

PATHOLOGY AND NATURAL BEHAVIOR

Almost any tumor can occasionally affect the perianal region, including lymphoma, soft tissue sarcoma, squamous cell carcinoma, melanoma, leiomyoma, transmissible venereal tumor, and mast cell tumor. However, the most common tumors are those that arise from the sebaceous glands of the perineum. The histologic distinction between adenomas and adenocarcinomas may not always be clear, and clinically there may be an intermediate condition called invasive perianal adenoma, which may look benign under the microscope yet be invasive in the patient.

Perianal adenomas follow a benign clinical course. The tumors are slow-growing, and although local disease may be extensive, metastasis does not occur.[1,4,5]

Perianal adenocarcinoma is generally associated with a low rate of metastasis (15%) at the time of original diagnosis.[8] It has been suggested that metastasis is most likely to develop later in the course of disease as the primary tumor becomes larger and more invasive.[8] The most frequent site is the regional sublumbar lymph nodes, including the sacral, hypogastric and iliac nodes.[1,8] Distant metastases affect lungs, liver, kidney and bone.[5,8] These tumors tend to be more rapidly growing, fixed, and firmer than the benign adenomas.

Anal sac apocrine gland adenocarcinoma is distinct from perianal sebaceous gland adenocarcinoma clinically and histologically. The metastatic potential of this tumor is well established with reported rates ranging from 46% to 96% at the time of initial presentation.[4,11-14,18] Regional sublumbar lymph nodes are by far the most common site of metastasis early in the course of disease.[13,14,18] The primary anal sac tumor may be as small as 1 cm with greatly enlarged metastatic nodes. Distant metastatic sites, including lungs, liver, spleen, bone, and less commonly heart, adrenal glands, pancreas, kidneys, or the mediastinum, may rarely develop later.[4,10,11,13] The association between anal sac apocrine gland adenocarcinoma and paraneoplastic hypercalcemia of malignancy, mediated by tumor secretion of parathyroid hormone-related peptide,[19,20] has been well documented.[11-15,21,22] Incidence of hypercalcemia is approximately 27% based on an analysis of 113 dogs with anal sac adenocarcinoma.[14]

Experimental immunohistochemical studies have attempted to elucidate possible molecular mechanisms of canine perianal tumorigenesis. In one study using a single polyclonal antihuman antibody, nuclear p53 accumulation was detected in 50% (8 of 16) of perianal sebaceous gland adenocarcinomas evaluated, suggesting that overexpression of a mutated p53 tumor suppressor protein may play a role.[23] Growth hormone has been detected immunohistochemically in 96% (23 of 24) of perianal adenomas and 100% (5 of 5) of perianal sebaceous gland adenocarcinoma.[24] The authors of that report suggested that growth hormone may be a mediator or contributor to tumor growth or evolution.

HISTORY AND CLINICAL SIGNS

The history for benign perianal adenoma is that of a slow-growing (months to years) mass or masses that are nonpainful and usually asymptomatic. These may be single, multiple, or diffuse (similar to generalized hyperplasia or hypertrophy of the perianal tissue) (Figure 21-27).[1] Most occur on the hairless skin area around the anus, although they may extend to haired regions and can develop on the prepuce, scrotum, or tail head (stud tail or "caudal tail gland").[1] Benign lesions may ulcerate and become infected but are rarely adherent or fixed to deeper structures.[1] They are usually fairly well circumscribed, on average 0.5 to 3 cm in diameter, and elevated from the perineum.[1]

The male perianal adenocarcinoma may look similar to adenomas but tends to grow more rapidly, be more firm, become ulcerated, adhere to underlying tissues, recur following surgery, and will generally be larger than their benign counterpart.[8] Obstipation, dyschezia, or perianal pain/irritation can be seen with larger masses.[8] Rarely, signs are related to obstruction of the
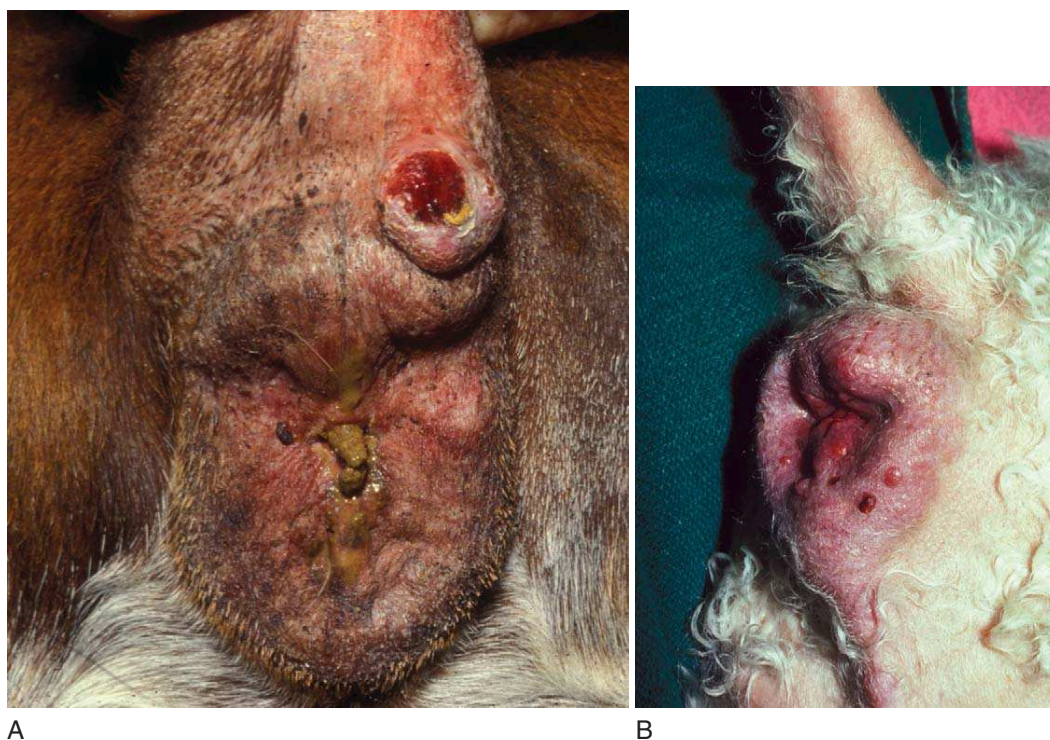
Figure 21-27

A, Typical small and ulcerated perianal adenoma can be seen at 1 o'clock. Treatment with castration and cryosurgery was curative. **B,** Diffuse 360° involvement of the perianal region with perianal adenoma. Aggressive resection or cryosurgery should not be performed but rather castration, a waiting period of several months for partial regression, and the local treatment for residual disease were applied.

pelvic canal by lymph node metastasis. Tumors can be multiple.[8] Castrated males with new or recurrent perianal tumors should raise the suspicion for malignant rather than benign disease, since adenocarcinomas are not hormonally dependent.

Clinical signs of dogs with anal sac adenocarcinoma are often referable to either the presence of the primary mass (perianal discomfort, swelling [Figure 21-28], bleeding or scooting), to hypercalcemia (polyuria, polydipsia; anorexia; lethargy), or to obstruction of the pelvic canal by sublumbar nodal metastasis (tenesmus; constipation).[13] In as many as 39% of dogs, the primary tumor can be an incidental finding on physical examination.[14] Rarely, regional bone metastasis or direct extension of tumor from sublumbar lymph nodes into the lumbar vertebrae with associated pain or fracture may be seen.

DIAGNOSIS AND STAGING

In the intact male with suspected perianal adenoma, a routine geriatric workup prior to anesthesia is desirable. Thoracic radiographs to evaluate for lung metastasis may not be cost effective unless indicated for other cardiopulmonary evaluation given the benign biologic behavior of these tumors. Evaluation of regional lymph node size is indicated if one suspects perianal adenocarcinoma based on signalment (neutered male), recurrent disease, or physical examination characteristics of malignancy (Figure 21-29). Ultrasonographic evaluation of sublumbar and pelvic lymph nodes is a superior staging tool in comparison to plain radiography, which can both under- and overestimate lymph node disease.[25] Although distant metastasis is uncommon, thoracic radiographs to evaluate for lung metastasis are recommended. Fine-needle aspiration and cytology to differentiate benign from malignant tumors in the male can be unrewarding, although they are helpful in ruling out other forms of cancer or mass development. Tissue biopsy is necessary to make the distinction in most cases, and the most important histologic criteria supporting a diagnosis of perianal sebaceous gland adenocarcinoma is invasiveness of tumor cells into surrounding tissue.[26] Disorderly arrangement of cells, increased nuclear pleomorphism, and increased numbers of mitotic figures also favor a diagnosis of malignancy.[26]

Figure 21-28

An anal sac adenocarcinoma in a female dog. Note the typical 4 o'clock position.

Figure 21-29

A Lateral radiograph of the caudal abdomen in a male dog with perianal adenocarcinoma. Note the metastatic involvement of sublumbar/iliac lymph nodes (*arrows*) and downward displacement of large bowel.

Figure 21-30

Fine needle aspirate cytology of an anal sac adenocarcinoma (400×). Typical epithelial clustering with bland, uniform morphology that belies its malignant potential.

Since dogs with anal sac adenocarcinoma may present with signs unrelated to perianal disease (i.e., polyuria, polydipsia due to hypercalcemia), assessment of animals with suspicious clinical signs requires a careful rectal examination, including palpation of the anal sacs and evaluation for possible lymphadenopathy. Definitive diagnosis must be made cytologically or histologically, but a strong presumption of anal sac adenocarcinoma can be made for animals in which rectal examination identifies the presence of a mass in the area of the anal sacs. This tumor has a characteristic cytologic appearance and is made up of polyhedral cells that have uniform round nuclei and light blue-gray, slightly granular cytoplasm (Figure 21-30). Features of malignancy may be subtle. In addition to suggesting cancer in the anal sacs, a fine needle aspirate is valuable in ruling out infection or inflammatory disease of the anal sac. The clinician should be aware that anal sac tumors can also be secondarily infected or inflamed, however. Staging of disease includes thoracic radiographs for detection of pulmonary or mediastinal involvement, as well as abdominal ultrasound to evaluate the size of regional lymph nodes and the echogenicity of other abdominal organs, especially liver and spleen. Computed tomography (CT) may yield a more complete assessment of abdominal involvment. In rare instances, pulmonary metastasis can be present without obvious regional lymph node disease. Lameness or bone pain should be evaluated with radiography or nuclear scintigraphy to rule out bone metastasis. Workup also includes complete blood count, serum chemistry panel, and urinalysis. Hypercalcemia of malignancy can result in significant renal damage, which may modify prognosis and anesthetic risk. Depending on the level of hypercalcemia and renal function, aggressive medical management may be in order prior to surgery (see Chapter 5).

Careful rectal examination should be performed on all dogs with perianal tumors to detect the clinical degree of fixation prior to surgery, the probability of resection and the risk of postoperative complications, particularly incontinence.

THERAPY AND PROGNOSIS

Due to the hormonal dependence of perianal adenomas, the vast majority of these tumors will regress (at least partially) following castration, and recurrence is uncommon.[5] Surgery is recommended in males with ulcerated tumors or recurrence, as well as in females with typical small and focal masses.[5] For diffuse or large benign lesions situated on or in the anal sphincter, castration followed by an observation period of several months to allow reduction in tumor volume may permit safer and easier mass removal. This will only be effective for benign lesions that are hormone dependent. Over 90% of male dogs will be cured with castration (male) and mass removal.[1,5] Adenomas can be excised with minimal margins. In addition to standard surgical techniques, mass removal can be achieved using cryosurgery or carbon dioxide laser, which should only be used for focal lesions less than 1 to 2 cm in diameter.[27,28] Use of either of these techniques precludes evaluation of the surgical margins for completeness of excision, which may be acceptable for benign masses; however, as the most consistent criteria for malignancy is invasiveness, lack of margin assessment is not without risk. Hyperthermia and radiation therapy have also been used successfully.[29,30] The cost, added morbidity, and limited availability of these techniques makes them a poor alternative to standard blade excision. Adenomas may also regress following estrogen therapy; however, its use is contraindicated by the risk of myelosuppression. Estrogens should only be considered in dogs with clinically significant tumors whose owners refuse castration or anesthesia. Chemical forms of castration, currently under development, may be effective in the future.

Perianal sebaceous gland adenocarcinoma is more locally invasive than its benign counterpart and generally does not respond to castration.[5] Aggressive surgical removal with adequate margins is indicated. Removal of half or more of the anal sphincter is possible with only rare transient loss of continence. Recurrent disease becomes more difficult to resect. Unfortunately, most perianal adenocarcinomas are not suspected or known until after a conservative resection, which contaminates further tissue planes, making the second resection problematic. Due to common local recurrence, this tumor is difficult to cure and may require numerous palliative resections over several years. Preoperative diagnosis of malignancy (i.e., incisional biopsy) and more aggressive initial resection should improve local control. The utility of postsurgical radiation therapy should also improve local control; however, data are lacking on this approach.

In a series of 41 dogs with perianal adenocarcinoma, stage of tumor had a significant influence on disease free interval and overall survival.[8] Tumors less than 5 cm in diameter (T2) were associated with 2-year tumor control rates in excess of 60%, suggesting that surgical removal of these masses at an early stage is relatively successful with respect to disease control. Fifteen percent of dogs had evidence of metastasis at the time of diagnosis, which related negatively to survival (Figure 21-31).[8] Median survival time for dogs with lymph node or distant metastasis was 7 months; however, aggressive treatment was not attempted in five of six cases.[8] If present, regional lymph node metastasis may be excised. Large nodal volume is not a contraindication to caudal abdominal exploratory and lymphadenectomy, as some nodes "shell out" readily while others are invasive. Enlarged lymph nodes should be considered for removal especially if they are causing obstruction of the pelvic canal. The use of radiation or chemotherapy, including actinomycin D, has been reported anecdotally, but their role in local or distant control is largely undefined.[8,31] Controlled clinical trials have not been performed.

Apocrine gland adenocarcinoma of the anal sac is generally locally invasive, and aggressive surgical excision is recommended. Recurrence rate is high after marginal surgery alone.[11,13] Complete resection of large perianal malignancies is difficult due to proximity to rectum and poor definition of perineal tissue to define an adequate margin. Metastasis is present at the time of diagnosis in approximately 50% to 80% of cases,[10,11,13,14,18] with regional lymph nodes being the most common site. Enlarged lymph nodes should be considered for removal, especially if they are causing obstruction of the pelvic canal or contributing to hypercalcemia. Radiation or chemotherapy may be used adjuvantly or as the sole treatment.[18,32] Omentalization has been proposed as an alternative to aggressive surgical resection for palliation of rare cystic nodal metastasis based on long-term palliation observed in one case treated in this manner.[33]

An analysis of 113 dogs with anal sac adenocarcinoma in varying stages of disease treated with differing forms of therapy reported a median overall survival time of 544 days (18 months) for all dogs.[14] Dogs that underwent surgery as part of their treatment had significantly improved survival (median, 548 days) than those that did not.[14] These results are in contrast to smaller earlier studies in which median survival times are between 6 and 12 months.[11,13,15] Proposed reasons for this improvement include earlier tumor recognition, more aggressive surgical and adjuvant treatment, and improved efforts at palliative care.[14] The longest survival for dogs with this disease is reported for combination surgery, curative-intent radiation, and mitoxantrone chemotherapy.[18]
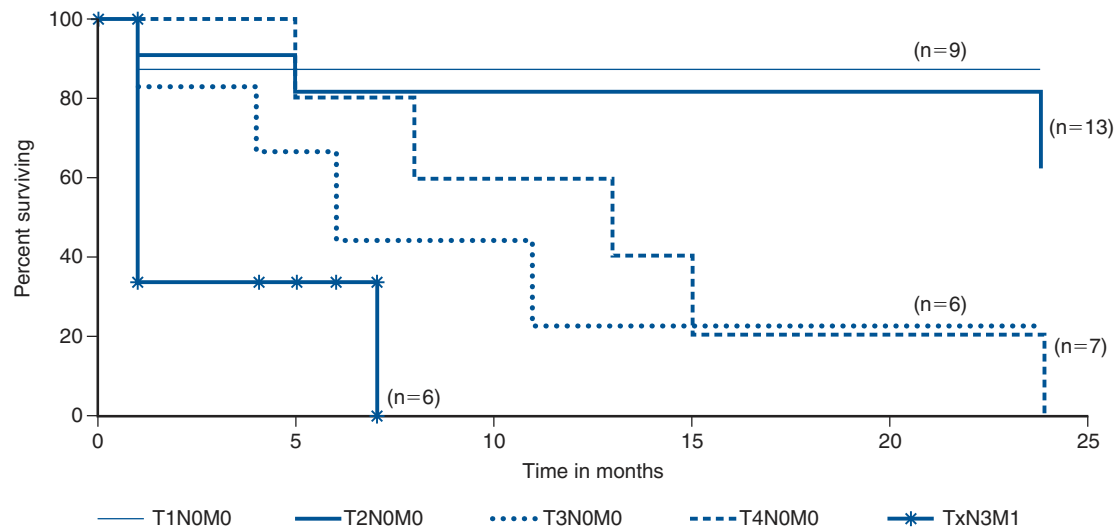
Figure 21-31

Survival duration in 41 male dogs with perianal adenocarcinoma based on stage. Note that dogs with small tumors (T1 or T2) without lymph node involvement will do well after aggressive removal of the primary. T1 = tumor <2 cm, T2 = tumor 2 to 5 cm, T3 = tumor >5 cm, T4 = tumor invading other structures such as fascia, muscle, or bone; N0 = no evidence of regional lymph node involvement, N3 = fixed lymph nodes; M0 = no evidence of distant metastasis, M1 = distant metastasis detected.

A review of 15 dogs treated in this manner suggested median event-free survival of 287 days (9 months) and an overall survival of 956 days (31 months).[18] The therapy was associated with self-limiting acute radiation complications in all dogs including mild to severe moist desquamation, colitis, and perineal discomfort lasting 2 to 4 weeks after therapy (Figure 21-32). More significant, however, more than half the cases treated this way experienced clinically relevant chronic radiation complications such as tenesmus, rectal stricture, and colitis. Incidence of chronic complications was similar to that reported in a review of late complications of pelvic irradiation.[34] None of the complications in the anal sac adenocarcinoma series was reported as life threatening, and all dogs maintained reasonable quality of life as assessed by pet owners. Measurable responses were seen in the gross disease setting; however, the relative importance of radiation versus mitoxantrone chemotherapy is not clear from this study.[18] Other equally staged and treated case series with different defined treatments are not available for comparison, making strong statements on "the best" treatment and expected morbidity and prognosis difficult to support. The platinum drugs, cisplatin and carboplatin, and actinomycin D have shown limited antitumor activity in a small number of dogs with this tumor.[13,31]

There is discordance regarding the prognostic significance of hypercalcemia of malignancy accompanying this tumor type. Hypercalcemia has generally been associated with shorter survival;[11,14] however, other studies have not

Figure 21-32

A white shepherd dog 4 months after multimodality therapy for anal sac adenocarcinoma. Therapy included primary excision, mitoxantrone chemotherapy, and radiation therapy to the perianal tissues and the sublumbar lymph node area. Note the denuded perianal tissues and moist desquamation secondary to radiation therapy.

supported this finding.[13,18] Complete or near-complete removal of the tumor results in reversal of hypercalcemia. Return of hypercalcemia after therapy usually signals recurrence or metastasis. The presence of pulmonary metastasis and primary tumors greater than 10 cm^2 are related to less favorable survival times.[14]

COMPARATIVE ASPECTS[35]

No similar hormonally dependent perianal disease state exists in humans. The most common cancer of the anal margin is squamous cell (epidermoid) carcinoma. These tumors arise from the junction of haired-skin and mucous membrane of the anal canal. Risk of developing cancer in this location is positively correlated with sexual activity. Precancerous changes (dysplasia) in the epithelium of the anal canal may precede tumor development. Regional lymph nodes are the most common site of metastasis. Size and degree of invasion of the primary tumor, as well as lymph node status, are the important prognostic factors. Surgical excision is the treatment of choice for smaller tumors. If excision would compromise sphincter function, if the tumor is large, or if the nodes are positive for metastasis, combined modality therapy including radiation and chemotherapy using 5-FU and mitomycin C are recommended. Overall, mean 5-year survival is approximately 63%.

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CHAPTER 22

Tumors of the Respiratory System

SECTION A
Cancer of the Nasal Planum

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Cancer of the nasal planum is rare in the dog and relatively common in the cat. The development of squamous cell carcinoma (SCC) has been correlated with ultraviolet light exposure and lack of protective pigment.[1] It classically is seen in older, lightly pigmented cats.

PATHOLOGY AND NATURAL BEHAVIOR

By far the most common cancer is SCC. Depending on when the biopsy is performed, the tumors may be reported as carcinoma *in situ*, superficial SCC, or deeply infiltrative SCC. They may be very locally invasive but only rarely metastasize.

Other cancers reported in the nasal planum are lymphoma, fibrosarcoma, hemangioma, melanoma, mast cell tumor, fibroma, and eosinophilic granuloma. Immune-mediated disease may manifest as erosive or crusty lesions on the nose, but it is rarely proliferative, and other sites on the body usually are affected. Immune-mediated disease probably is not a contributing factor in tumor development.

HISTORY AND CLINICAL SIGNS

Invasive SCC usually is preceded by a protracted course of disease (months to years) that progresses through the following stages (Figure 22-1): crusting and erythema, superficial erosions and ulcers (carcinoma *in situ* or early SCC) and, finally, deeply invasive, erosive lesions. In cats SCC usually originates on the cornified external

surface of the nasal planum, whereas in dogs it often occurs in the mucous membrane of the nostril or the external planum. Associated lesions on the eyelid, preauricular skin, and ear pinna may be seen in cats if these sites lack pigment. Patients often have been treated with corticosteroids or topical ointments, with minimal response.

DIAGNOSTIC TECHNIQUES AND WORKUP

For erosive or proliferative lesions, a deep wedge biopsy should be done to determine the degree of invasion and the histologic type of disease. These biopsies require a brief general anesthetic because of the sensitivity of the nasal planum. Hemorrhage can be temporarily profuse and usually requires one or two sutures to appose the edges. Cytologic scrapings and superficial biopsies are of little value, because they frequently reveal only inflammation, which may accompany both cancer and noncancerous conditions. Lymph nodes are rarely involved except in very advanced disease, and thoracic radiographs are almost invariably negative for metastasis. Regional radiographs generally are unrewarding. Computed tomography (CT) and magnetic resonance imaging (MRI) have proved to be valuable staging tool in dogs with SCC of the nostril. They can help define the posterior extent of the tumor and guide the posterior level of resection or the posterior extent of the radiation field.

THERAPY

Limiting exposure to the sun or tattooing to add pigment protection may prevent or arrest the course of the preneoplastic disease. Topical sunscreens are easily licked off and rarely help. Maintaining the tattoo is very difficult when inflammation and ulceration are present, because it is rapidly removed by macrophages. Even under the best of circumstances, tattooing must be
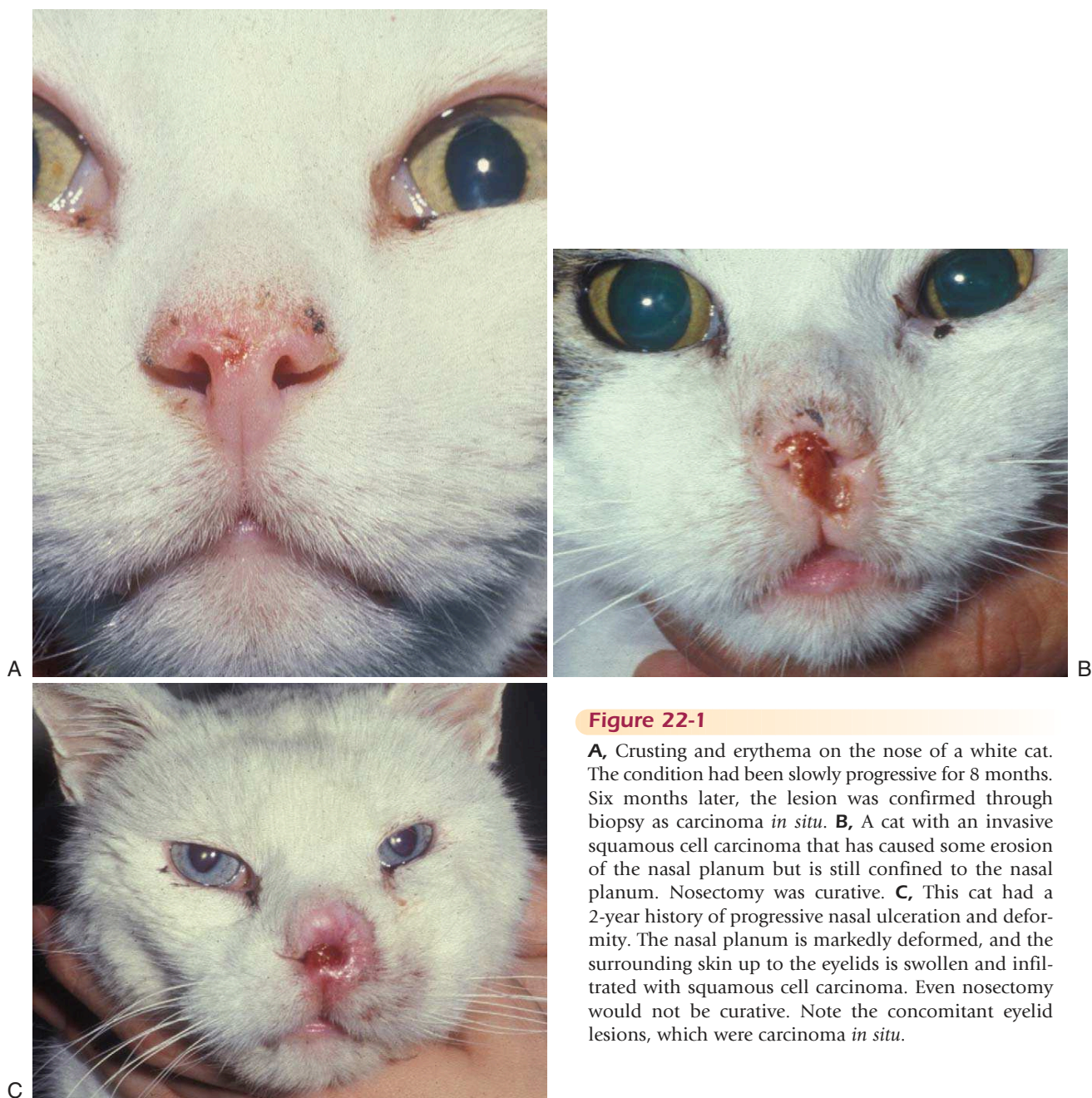
Figure 22-1

A, Crusting and erythema on the nose of a white cat. The condition had been slowly progressive for 8 months. Six months later, the lesion was confirmed through biopsy as carcinoma *in situ*. **B,** A cat with an invasive squamous cell carcinoma that has caused some erosion of the nasal planum but is still confined to the nasal planum. Nosectomy was curative. **C,** This cat had a 2-year history of progressive nasal ulceration and deformity. The nasal planum is markedly deformed, and the surrounding skin up to the eyelids is swollen and infiltrated with squamous cell carcinoma. Even nosectomy would not be curative. Note the concomitant eyelid lesions, which were carcinoma *in situ*.

repeated regularly. Attempts to increase epithelial differentiation with synthetic derivatives of vitamin A generally have been unsuccessful for advanced disease but may be of help in reversing or limiting the growth of preneoplastic lesions.[2,3]

Squamous cell carcinoma and probably other neoplasms as well fall into two general categories: superficial, minimally invasive disease and deeply infiltrating disease. Superficial cancers can be managed effectively by almost any method, including cryosurgery, lasers,[4] phototherapy,[5] intralesional carboplatin chemotherapy,[6] intralesional carboplatin and radiotherapy,[7] hyperthermia, and irradiation.[8-12] A distinct disadvantage with these techniques is the inability to obtain a surgical margin by which to document the adequacy of treatment. Deeply invasive cancer is generally resistant to these treatments. Radiation therapy, in particular, which would have the greatest chance of preserving the cosmetic appearance of the nose, has had poor local control rates for larger and more invasive SCC in the dog and the cat.[10,11,13] Expectations for radiation with other tumor types must be extrapolated from results achieved in more conventional sites.
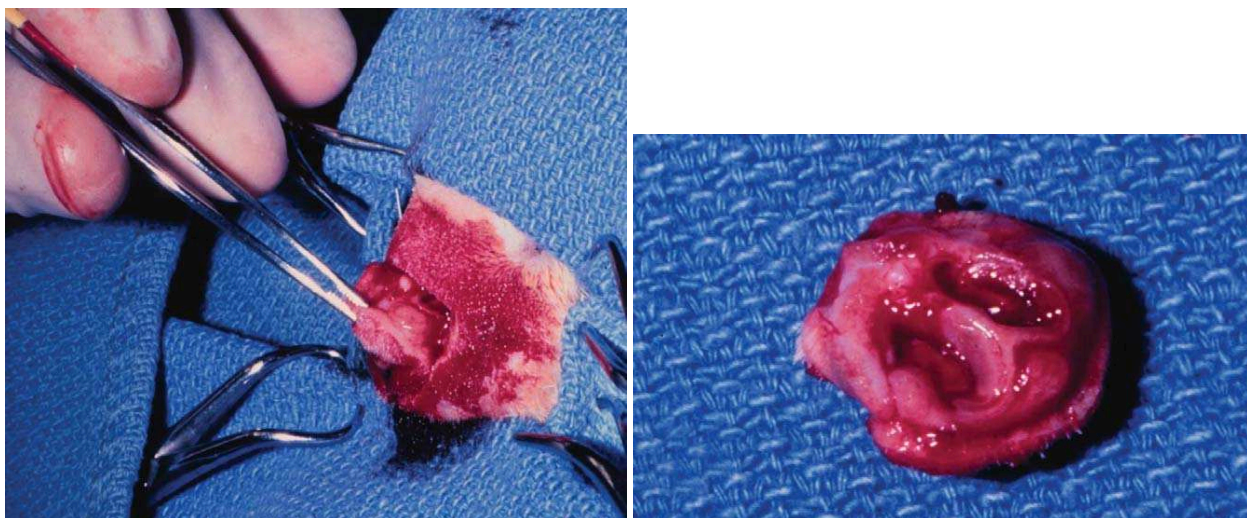
Figure 22-2

Operative view of resected nasal planum in a cat with invasive carcinoma that was confined to the nasal planum.

In the cat, invasive cancer of the nasal planum can be completely excised with an acceptable cosmetic result.[14] The nasal planum is removed with a 360-degree skin incision that also transects the underlying turbinates (Figure 22-2). A single cutaneous purse-string suture of 3-0 nylon is placed to pull the skin into an open circle (1 cm in diameter) around the airways. The purse-string must not be pulled too tight, or it may heal across the airways. The site subsequently crusts and scabs over. The scab is removed at suture removal (this often requires sedation), and healing of the skin with two patent airways ("nostrils") is complete by 1 month. An occasional problem with nosectomy is stricture of the combined nasal orifice. This can be frustrating and has been variously managed with wide skin excision and removal of the nasal septum rostrally, laser ablation, rubber stents, or permanent placement of a stainless steel intraluminal expansile stent.[15] Functional and cosmetic results are good in the cat (Figure 22-3), and fair to good in the dog (Figure 22-4). This procedure probably is the treatment of choice for invasive lesions that have not spread extensively to the lip or surrounding skin. The author has found that adjuvant radiation therapy has been successful if the margins of removal are incomplete. Combined removal of the premaxilla and nasal planum has been reported in the dog.[16,17]

PROGNOSIS

The outlook for SCC is good for early, noninvasive disease. However, the development of new sites of
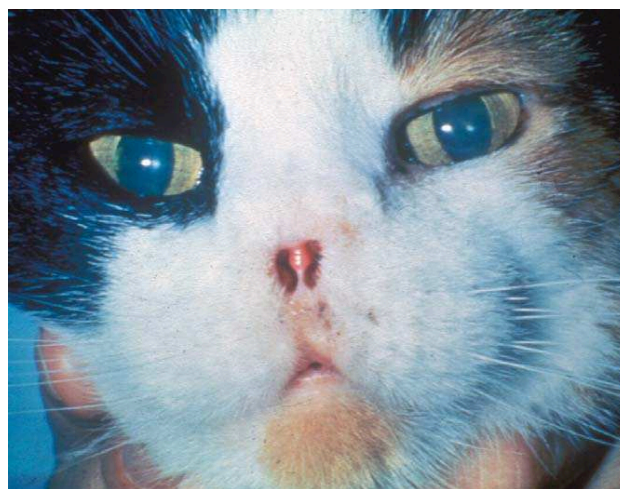
Figure 22-3

One-year postoperative view of a cat after nosectomy for squamous cell carcinoma. Note the well-healed skin around patent airways. This cat remains free of disease 28 months after surgery.

neoplasia on other areas of the planum after localized treatment is common, because the underlying causes are not reversed.[18] Later stage disease can be cured with aggressive surgery but is poorly responsive to most other treatments.[13,14,16] More than 80% of cats with invasive SCC of the nasal planum treated with wide resection (nosectomy) are free of recurrent disease at 1 year.[4,14] Newer reports of proton irradiation[12] and
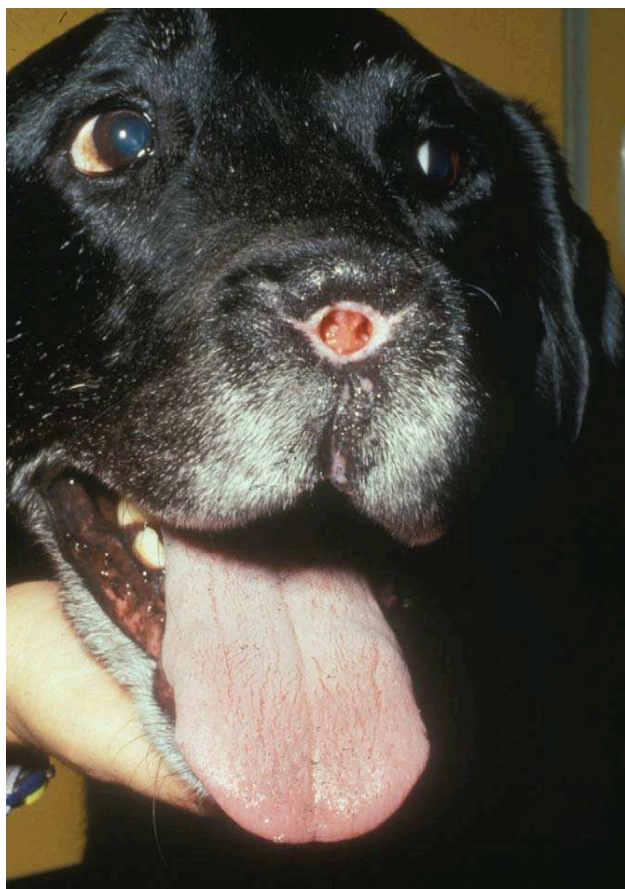
Figure 22-4

Two-year postoperative view of a dog after nasal planum resection for squamous cell carcinoma.

irradiation with intralesional chemotherapy[7] suggest control rates in cats exceeding 60%. In a study of nosectomy (with or without removal of the pinnae) in 38 cats, the median survival time exceeded 22 months.[4] Photodynamic therapy was effective in treating nine of 16 cats with SCC of the nose, but the lesions had to be small and minimally invasive for a good result.[5] In dogs, the local recurrence rate after nosectomy is low.[13,17] Delayed lymph node metastasis (longer than 1 year after surgery) in dogs with SCC has been successfully treated with lymphadenectomy. Because SCC rarely metastasizes from the nasal planum, even untreated animals with advanced cancer can live a long time, albeit with an ulcerated and deforming cancer.

COMPARATIVE ASPECTS[19]

As in the cat, cancer of the human nasal skin and nasal vestibule (anterior entrance to the nasal cavity) may be induced by exposure to ultraviolet light. Lack of protective pigment is also a contributing factor in humans. SCC of the vestibule is treated with irradiation (interstitial or external beam) or surgery. Surgery generally involves resection of the nasal skin and cartilage, followed by reconstruction using composite ear skin and cartilage, nasolabial flaps, or a prosthesis. Local control generally is good.

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SECTION B
Cancer of the Larynx and Trachea

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Cancer in either the larynx or the trachea is rare in dogs and cats.[1,2] Young patients with active osteochondral ossification sites are at higher risk for benign tracheal osteocartilaginous tumors, which grow in synchrony with the rest of the musculoskeletal system.[1,3] Laryngeal oncocytomas also appear to occur in younger mature dogs.[4,5] No breed or gender predilection is known for either site.

PATHOLOGY AND NATURAL BEHAVIOR

Reported canine laryngeal tumors include rhabdomyoma (oncocytoma), osteosarcoma, extramedullary plasmacytoma,[4] chondrosarcoma, undifferentiated carcinoma, fibrosarcoma, mast cell tumor, adenocarcinoma, and squamous cell carcinoma.[7-9] Rhabdomyomas in the dog may be large, are minimally invasive, and do not appear to metastasize.[4,5,10,11] Most other laryngeal tumors are very locally invasive and have a significant metastatic potential. Feline laryngeal neoplasms most commonly are lymphoma, although squamous cell carcinoma and adenocarcinoma have been reported.[2,8,9,12,13] Benign tracheal tumors and cysts in cats are rare.[2,12,14]

Reported tracheal cancer includes lymphoma, chondrosarcoma, adenocarcinoma, and squamous cell carcinoma.[15] Tracheal leiomyomas and polyps have also been reported.[16-18] Several reports of benign tracheal osteochondral tumors exist in the literature.[3,19] These lesions grow from the cartilaginous rings and are composed of cancellous bone capped by cartilage. They may reflect a malfunction of osteogenesis rather than true cancer and are benign.[19] The larynx and trachea may be secondarily invaded by neoplasms such as lymphoma and thyroid adenocarcinoma.

HISTORY AND CLINICAL SIGNS

Patients with laryngeal tumors usually have a progressive change in voice or bark, exercise intolerance, or dysphagia. Patients with tracheal tumors usually have coughing and exercise intolerance. Because osteochondral lesions of young dogs grow at the same rate as the rest of the skeleton, symptoms become most noticeable during the skeletal growth spurt.

DIAGNOSTIC TECHNIQUES AND WORKUP

Laryngeal tumors usually can be biopsied under direct visualization. Small samples or cytology alone may yield false negative results.[2] Regional radiographs may reveal the lesion but are rarely necessary.[7]

Tracheal tumors offer more of a diagnostic challenge but can be biopsied with the use of fiberoptic instruments or a rigid bronchoscope (Figure 22-5). Alternatively, open surgical biopsy, often coupled with excision, can be performed. Plain radiographs or a tracheogram may reveal a mass narrowing the lumen (Figure 22-6). CT or MRI may aid localization of the lesion.

THERAPY

Benign laryngeal cancers, such as rhabdomyomas and cysts, can be removed successfully with preservation of function.[4,10,11,14] Complete laryngectomy with a permanent tracheostomy is another option used in humans, but it has had limited use in veterinary medicine.[9,20-24] Depending on their suspected radioresponsiveness, invasive cancers can be treated with irradiation to better preserve laryngeal function. Radiation should control lymphoma in the dog or cat larynx or trachea. Chemotherapy, with or without surgery, may also be effective.[25]

Tracheal tumors should be treated with resection, especially benign osteochondral tumors (Figure 22-7).[3,26] Full thickness removal with end-to-end anastomosis can easily be performed on up to three or four rings. Experimentally, up to 50% of tracheal length has been removed with successful closure.[27]
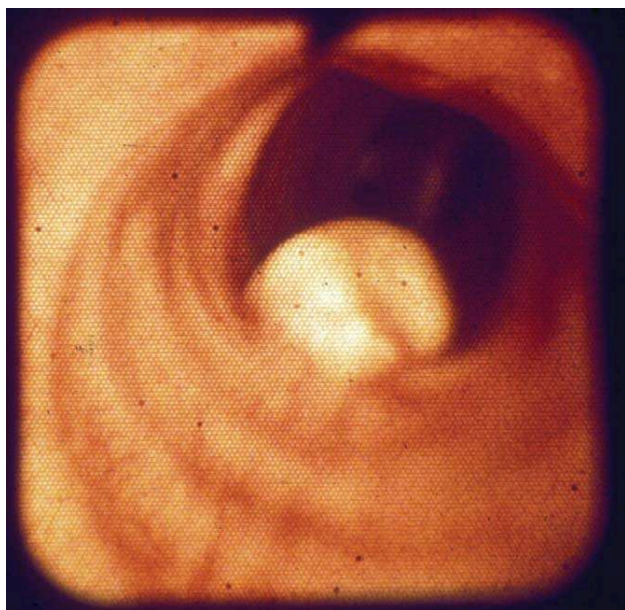
Figure 22-5

Fiberoptic view of tracheal osteochondroma in a 5-month-old dog.

Figure 22-6

Lateral thoracic radiograph of the dog in Figure 22-5. Note the intraluminal mass on the floor of the trachea *(arrows)*.

Phototherapy via bronchoscopy has been used successfully in humans for small lesions (carcinoma *in situ* or early carcinoma), but these lesions are only rarely recognized in the dog or cat.

PROGNOSIS

Benign lesions of the trachea and larynx have a good prognosis if they can be resected.[14] Most dogs with resectable rhabdomyomas live longer than 1 year and may be presumed cured.[4,10,11] Very limited information is available for malignancies, because very few have been treated and reported.[9,15] In one study in cats, the median survival time for 27 cats with a variety of laryngeal and tracheal lesions was 5 days, and only 7% were alive at 1 year.[2]

COMPARATIVE ASPECTS[28]

Laryngeal cancer is common in humans (2% of all cancers) and is related to smoking and alcohol consumption. The lesion is nearly always squamous cell carcinoma. Earlier detection prompted by voice changes make treatment more feasible. In humans the disease appears to progress through stages of development from dysphagia, to carcinoma *in situ*, to minimally invasive carcinoma, to invasive carcinoma. Of patients with

Figure 22-7

Resected tumor and associated tracheal cartilage rings of the dog in Figures 22-5 and 22-6. The tumor is bisected and has the typical appearance of cancellous bone with a cartilaginous cap. The patient recovered uneventfully from surgery and survived for longer than 5 years, when it was lost to follow-up.

carcinomas, 60% have local disease only, 30% have regional nodal metastasis, and 10% have distant metastasis. The disease is treated with surgery (partial or complete laryngectomy) or irradiation. Local control and cure rates are good to excellent.

Tracheal cancer independent of lung cancer is very rare in humans.

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SECTION C
Lung Cancer

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Compared to lung cancer in humans, primary canine lung cancer is very rare (1% of all cancers diagnosed). In a closed colony of normal beagles, primary lung cancer was detected in 9% of the dogs at necropsy. The median life span was 14 years.[1] Primary lung cancer is even more uncommon in the cat. Attempts to correlate urban living[2] and passive cigarette smoking as yet have not shown a convincing positive etiologic association.[3,4] However, dogs trained to smoke cigarettes (with or without concomitant asbestos instillation) through a tracheostomy develop lung cancer at a dramatically higher rate.[5,6] Plutonium[239] and other forms of radiation have been shown to induce lung cancer in normal dogs when inhaled as an aerosol.[7] The average age at diagnosis is 10 years, and no apparent gender or breed predilection has been noted in dogs. Older female cats are more commonly affected than male cats. Metastatic cancer to the lung is much more common than primary lung cancer in the dog and the cat.[8–10]

PATHOLOGY AND NATURAL BEHAVIOR

Almost all primary cancers of the lung are carcinomas, the most common being adenocarcinoma.[1,8,9] A monoclonal antibody to thyroid transcriptional factor is highly specific and moderately sensitive as a marker for canine pulmonary epithelial tumors.[9] Adenocarcinomas are further classified by location (e.g., bronchial, bronchoalveolar, or alveolar carcinoma). Most primary lung tumors are solitary, although a rare form of diffuse bronchioloalveolar carcinoma in a dog has been reported.[11] Carcinomas can be further graded as differentiated or undifferentiated, which correlates with the incidence of metastasis.[8,12] Squamous cell carcinoma is less commonly reported. Benign tumors and primary sarcomas are rare.[8,13,14] Lymphomatoid granulomatosis is a rare neoplasm of the lung.[15]

Metastasis can occur via lymphatics, through the airways, hematogenously, or transpleurally.[16] In both humans and animals, lung cancer seems to have a propensity for spreading to the central nervous system.[17-19] More than 50% of undifferentiated adenocarcinomas and more than 90% of squamous cell carcinomas metastasize.[8] Feline primary lung tumors were metastatic in almost 75% of cases in one study,[20] and a syndrome of multiple digital metastasis has been recognized in several reports (see Chapter 18). A feline bronchioloalveolar lung carcinoma cell line was recently characterized.[21]

HISTORY AND CLINICAL SIGNS

Most symptomatic animals have nonproductive coughing, exercise intolerance, or other respiratory signs that have been present for several weeks to months. An occasional animal may be brought for treatment peracutely secondary to hemothorax, pneumothorax, or malignant pleural effusion.[22]

Paraneoplastic syndromes, which are common in humans, are rare (or unrecognized) in animals, the most common being hypertrophic osteopathy.[16,17] When such a syndrome is present, the animal may be seen for lameness or swollen legs. A case of profound leukocytosis was associated with a lung carcinoma in a dog.[23] The leukocytosis resolved after resection of the tumor.

DIAGNOSTIC TECHNIQUES AND WORKUP

Theoretically, many diagnostic techniques are available to detect lung cancer, but only rarely are they indicated. Once a thoracic radiograph demonstrates a solitary lung mass, the next step generally is surgical removal; however, advanced imaging (CT) can aid the assessment of resectability and has documented use for predicting lymph node metastasis.[paoloni] The clinical dilemma is deciding which lung lesions are likely to be primary lung cancer and treatable with surgery, as opposed to those that should not be operated on or should be treated in another fashion. However, a strong presumptive diagnosis can be made before surgery.

Radiographs usually demonstrate a well-demarcated, spherical mass that usually is solitary. Primary or metastatic lung cancer occasionally has a cavitary appearance on radiographs.[24] The caudal lung lobes are most commonly affected (Figure 22-8). Multiple or miliary lesions may be seen but are less common.[11] Hilar lymphadenopathy is not often seen on radiographs, even though positive lymph nodes may be removed at surgery. Pleural effusion may also be detected, but it only rarely contains cancer cells.[25] Other imaging techniques (e.g., MRI and CT scans) may allow more accurate staging for resectability and detection of occult metastasis or lymph node enlargement.

Bronchoscopy can be performed, and tissue or brush biopsy may be of diagnostic value for centrally located lesions that extend into the bronchus.[26] Transtracheal or bronchoalveolar lavage also can be performed, but it is only rarely diagnostic except in diffuse lymphoma, for which clinicians should have other, simpler, and safer diagnostics at their disposal, negating the need for lavage.[27-30] Transthoracic fine-needle aspirates can be
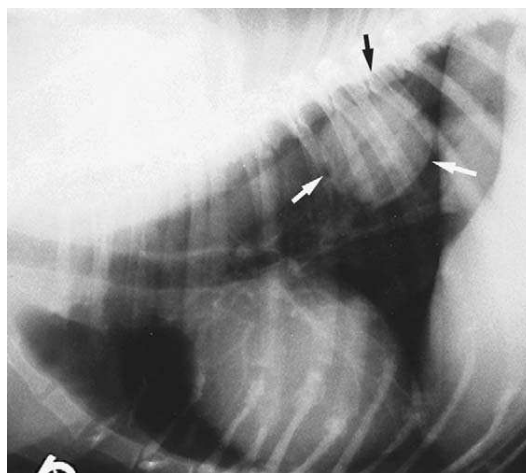
Figure 22-8

Lateral radiograph of a 12-year-old dog with a well-circumscribed mass in the caudal lung lobe. This mass was removed surgically by lobectomy and diagnosed as an adenocarcinoma. The patient died 26 months later of causes other than cancer, and necropsy revealed no evidence of cancer.

quite rewarding for larger lesions in a peripheral location; however, these tumors often have a necrotic center that may confuse interpretation.[31-34] One study suggested that transthoracic needle aspiration biopsy was 80% accurate in dogs and cats with diffuse interstitial disease, but the procedure had a 12% fatality rate.[35] Ultrasound-guided fine-needle aspiration of thoracic masses, including lung cancer, was found to be 91% diagnostic in one study.[36] However, bronchoscopy, tracheal lavage, and fine-needle aspiration often are unnecessary before surgery for solitary lesions because the treatment (i.e., lobectomy) is the same for all etiologies. Unless the owner wants an accurate diagnosis or prognosis before surgery, the diagnosis and treatment should be combined.

Pleural effusions, however, should be carefully assessed, because malignant pleural effusions have a very poor prognosis and may limit the value of surgical intervention. Thoracoscopy and CT imaging may be valuable tools for determining the etiology of pleural effusions.[37]

THERAPY

Once a diagnosis of a solitary lung mass has been made, with disease that is apparently confined to the thorax, and with a patient that can tolerate anesthesia, thoracotomy generally is the next step; however, CT for staging is becoming more common as a presurgical planning step. Thorascopic lung lobectomy recently was described in nine dogs.[38] Conversion to thoracotomy was necessary in four dogs because of poor visualization. Small masses away from the hilus may be reasonable candidates for thorascopic resection.[38]

A standard intercostal incision over the fourth to sixth interspace generally allows complete lung lobe removal of small to medium tumors and hilar lymph node biopsy. Pulmonary lymphatic mapping with technetium colloid and isosulfan blue has been described to locate sentinel nodes in normal dogs.[39] A midline sternotomy approach allows removal of larger tumors and better inspection of other lung lobes but makes lymph node biopsy more difficult. Partial lobectomy can be performed for very peripherally located tumors, but a complete lobectomy is the rule. Careful palpation of other lobes may reveal further nodules for removal. Stapling equipment (TA-55 or TA-90)* has allowed quick, secure lobectomy in most patients but is not required (Figure 22-9).[33,40]

Other methods of therapy, such as irradiation and chemotherapy, have been largely untried. Normal lung tissue generally does not accept the doses of radiation required to kill tumor cells without serious consequences,

such as fibrosis. However, newer methods of irradiation, including intensity-modulated radiation therapy (IMRT), are used routinely in physician-based medicine and have been applied on a limited basis in dogs with lung tumors; these are likely to become more widely available for clinical use in the future. In a very limited series of cases, multidrug chemotherapy with vindesine (with or without cisplatin) may have shown some benefit.[33,41] Vinorelbine (Navelbine) has been used in dogs with a variety of cancers, and partial responses were seen in two dogs with bronchioloalveolar carcinoma.[41] Use of this drug as an adjuvant for dogs with poor prognostic indices currently is undergoing evaluation and should be considered.[41] Inhalation chemotherapy has been described for animals with measurable lung cancer and for those in the adjuvant setting. Results are preliminary but encouraging, with acceptable toxicity.[42,43] Isolated lung perfusion with chemotherapy for unresectable disease has been tested in dogs and humans but is technically demanding and associated with significant toxicity.[44]

Malignant pleural effusions can be treated with systemic chemotherapy, intrapleural chemotherapy, or both with such agents as cisplatin, carboplatin, or mitoxantrone.[45] Alternatively, sclerosing agents (e.g., tetracycline or talc) have been used for palliative pleurodesis.[46]

Metastatic cancer to the lung is treatable on rare occasions.[47-50] The criteria for operating on a patient with metastasis are relative but include complete control of the primary cancer (the longer the better, but at least 300 days); no other known metastatic sites; prior exposure to "effective" chemotherapy that hopefully eradicated other disease sites; a "favorable histology" (this is not established in veterinary medicine but generally includes sarcomas); a slow radiographic diameter doubling time (longer than the 40-day doubling of tumor diameter); and fewer than three radiographically visible lesions. Of dogs that fit most of these criteria, one fourth to one third can expect longer than 1 year survival after metastasectomy (Figure 22-10).[50]

PROGNOSIS

The best prognosis is seen in dogs with solitary lesions of small diameter (less than 5 cm), negative lymph nodes, no malignant pleural effusion, and well-differentiated adenocarcinoma.[12,33] In this group of patients, more than 50% can be expected to live at least 1 year after surgery. Of the malignant cancers, adenocarcinomas have a better prognosis than squamous cell carcinoma, which often is diffuse at diagnosis. One study reported a mean survival time of 8 months for three dogs with squamous cell carcinomas and

*U.S. Surgical, Autosuture, Norwalk, Conn.
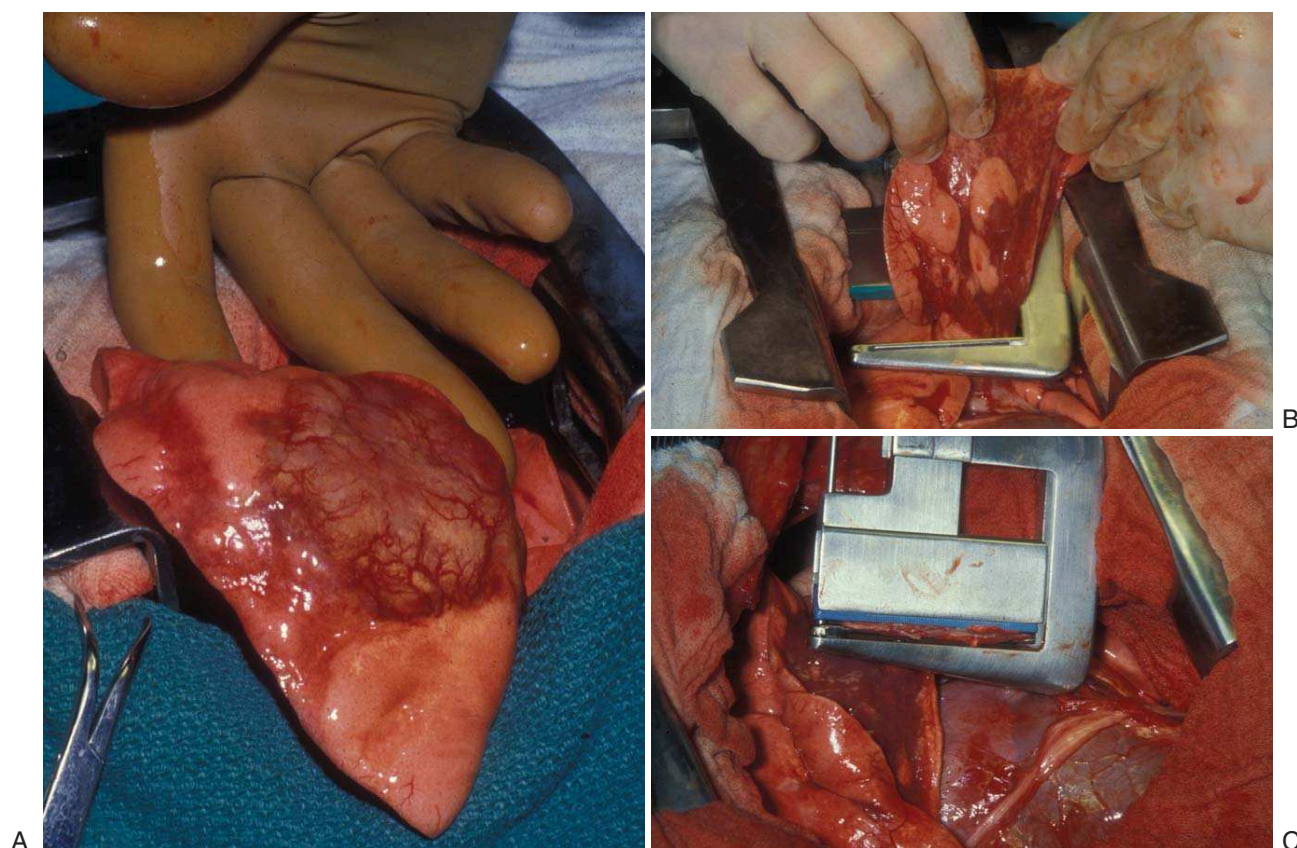
Figure 22-9

A, Operative view of lung cancer from the patient in Figure 22-8. Note the typical raised lesion with superficial neovascularization. **B,** The lung lobe is lifted up and surgical staples are placed at the level of the proximal mainstem bronchus. **C,** Firing the stapler releases a double row of B-shaped, stainless steel staples. The lung mass then is removed, and the instrument is released. The artery, vein, and bronchus are inspected for any small leaks, which are sutured.

19 months for eight dogs with adenocarcinomas.[33] Dogs with tumors in the periphery had better survival times than those with tumors that involved the entire lobe (mean survival time of 16 months versus 8 months). Similarly, patients with small tumors (less than 100 cm³, 5 cm diameter) had a mean survival time of 20 months, whereas those with large tumors (more than 100 cm³) had a mean survival time of 8 months.[33] In another case series, patients with lymph node involvement had a median survival time of 60 days, whereas dogs without lymph node involvement had a median survival time of 12 months.[12]

A recent, single institution study of primary lung tumors in 67 dogs showed a significant correlation between survival and the histologic type and grade, tumor stage, presence of clinical signs at presentation (ominous), and metastasis to regional nodes (Figure 22-11).[51] The overall median survival for all dogs was 1 year. Two perioperative deaths occurred, both in patients with incompletely resected disease. Dogs with low grade tumors had a median survival of 16 months, and those with high grade tumors had a median survival of 6 months. Dogs with no clinical signs had a median survival of 18 months, and those with clinical signs had a median survival of 8 months. The tumor stage (T) was also prognostic for medial survival time (T_1 tumors, 26 months; T_2 tumors, 7 months; and T_3 tumors, 3 months). Dogs in which the lymph nodes tested negative survived a median of 15 months, and dogs in which the nodes tested positive survived a median of 1 month. Patients with low grade papillary carcinoma had a median survival of 17 months, whereas in those with all other cell types, the median survival was 1.5 months.[51]

Hypertrophic osteopathy, if present, can be expected to resolve after complete removal of the lung mass.
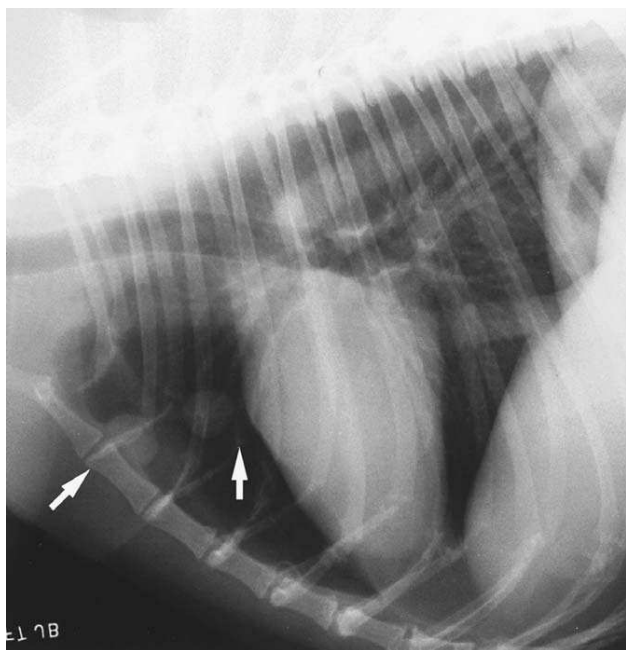
Figure 22-10

Lateral radiographs of a 10-year-old Irish setter that had undergone forelimb amputation for osteosarcoma 8 months previously. Note the two spherical lesions in the apical lung lobes *(arrows)*. The diameter of these lesions increased only 25% in 40 days, and they were removed surgically through a partial lobectomy. This patient did well for 8 more months, when a rib metastasis was noted. No further lung cancer was noted.

COMPARATIVE ASPECTS[52]

Primary lung cancer develops in more than 100,000 people each year in the United States. It is the leading cause of cancer death in men and women over 35 years of age, and it is strongly associated with smoking. Bronchial or bronchoalveolar carcinomas and bronchial mucinous gland carcinomas account for more than 90% of lung cancers. The most common histologic types, in order, are adenocarcinoma, squamous cell carcinoma, small cell (oat cell) carcinoma, and large cell carcinoma. Paraneoplastic syndromes are common and diverse, seen in more than 50% of cases. Resection is the treatment of choice for most lesions. If resection for cure can be performed, up to 30% of non–small cell cancer patients may survive 5 years. Small cell carcinoma historically has had the worst prognosis. Although small cell carcinoma at first may respond well to chemotherapy or irradiation, long-term control is poor. Radiation generally is reserved for nonresectable tumors or for palliation.

MISCELLANEOUS CONDITIONS OF THE LUNG

Canine Pulmonary Lymphomatoid Granulomatosis[15,53,54,55]

In a series of seven cases, canine pulmonary lymphomatoid granulomatosis (PLG) affected young to middle-aged dogs, with no breed or gender predilection noted.[15] The most consistent laboratory abnormalities were circulating basophilia (six cases) and leukocytosis (four cases). Radiographic changes included lung lobe consolidation or large pulmonary granulomas (six cases) and tracheobronchial lymph node enlargement (six cases) (Figure 22-12). Transthoracic fine-needle aspirates yielded evidence of eosinophilic and neutrophilic inflammation and were not diagnostic. All the dogs tested negative for heartworm. The major differential diagnoses were heartworm granulomas and either metastatic or primary lung cancer. Definitive diagnosis was based on histologic examination of involved tissues (generally by means of an open biopsy). This examination characteristically revealed angiocentric and angiodestructive infiltration of the pulmonary parenchyma by large lymphoreticular and plasmacytoid cells, along with normal lymphocytes, eosinophils, and plasma cells. The infiltrate characteristically was centered around the small to medium arteries and veins. A less than ideal but practical approach to diagnosis is the response to therapy. Open lung biopsy by means of thoracotomy is not a low-risk procedure in dogs with diffuse lung disease.

To our knowledge, PLG is the only diffuse neoplastic primary lung disease that rapidly responds to chemotherapy. If the dog has a complete response in 1 or 2 weeks, the clinician may be able to assume a diagnosis of PLG. However, one report on PLG found only a moderate response to therapy.[53] Full characterization of this unusual disease awaits further study.

Treatment consisting of cytotoxic chemotherapy was attempted in five dogs.[15] The drugs used included cyclophosphamide, vincristine, and prednisone. A complete response was achieved in three of the five dogs, as evidenced by both clinical and radiographic resolution of signs (see Figure 22-12, *B*). These three animals remained in complete remission at 7, 12, and 32 months after diagnosis and initiation of therapy. Of the two remaining dogs, one was euthanized because of progression of signs after treatment with prednisone alone; the other, treated with combination chemotherapy, developed lymphoblastic leukemia 2 months after initiation of therapy. Two dogs were euthanized at the time of diagnosis.

The etiology of lymphomatoid granulomatosis is unknown, although the disorder may be a preneoplastic,

A

Grade 1
Grade 2
Grade 3

B

C
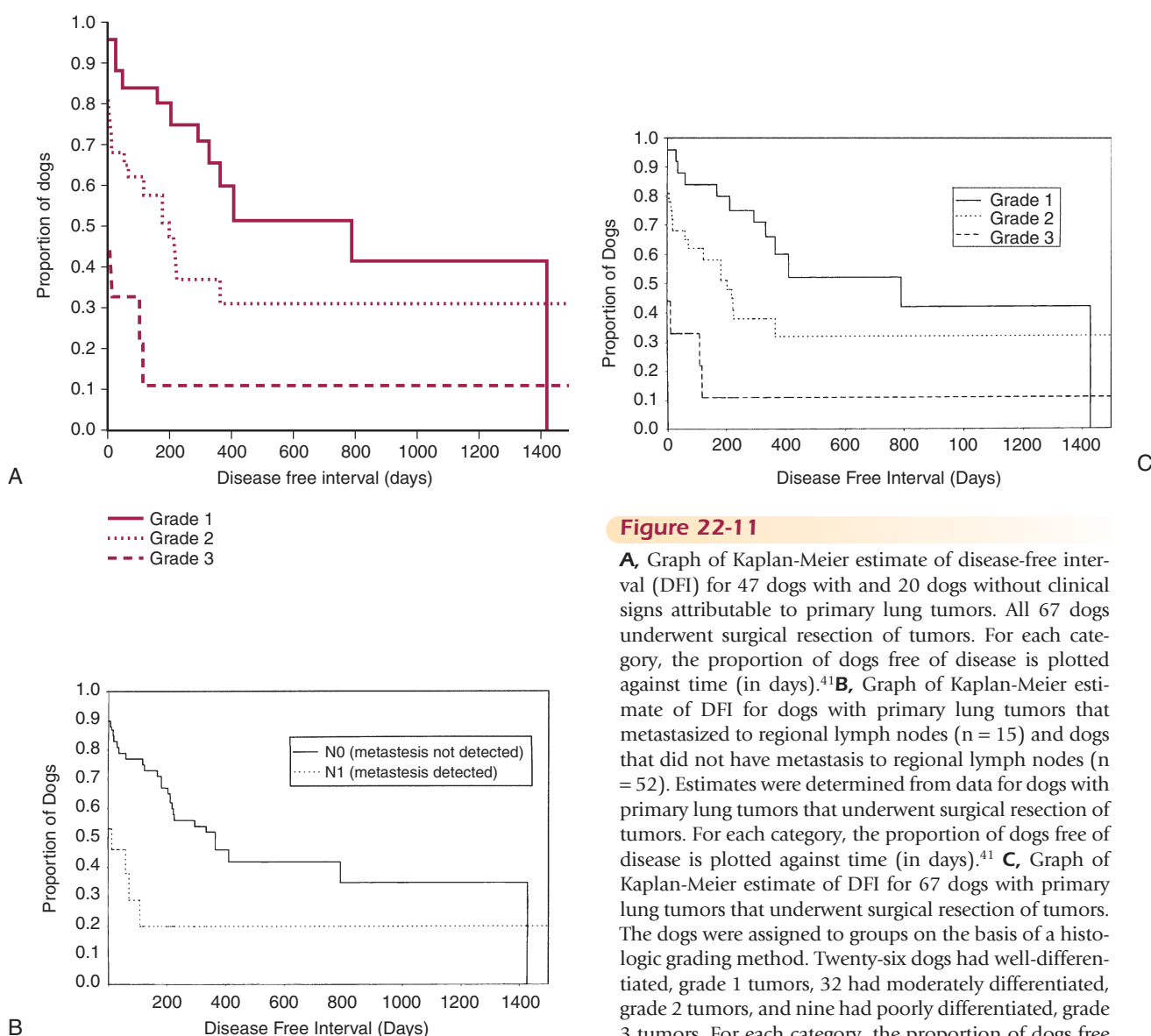
Figure 22-11

A, Graph of Kaplan-Meier estimate of disease-free interval (DFI) for 47 dogs with and 20 dogs without clinical signs attributable to primary lung tumors. All 67 dogs underwent surgical resection of tumors. For each category, the proportion of dogs free of disease is plotted against time (in days).[41] **B,** Graph of Kaplan-Meier estimate of DFI for dogs with primary lung tumors that metastasized to regional lymph nodes (n = 15) and dogs that did not have metastasis to regional lymph nodes (n = 52). Estimates were determined from data for dogs with primary lung tumors that underwent surgical resection of tumors. For each category, the proportion of dogs free of disease is plotted against time (in days).[41] **C,** Graph of Kaplan-Meier estimate of DFI for 67 dogs with primary lung tumors that underwent surgical resection of tumors. The dogs were assigned to groups on the basis of a histologic grading method. Twenty-six dogs had well-differentiated, grade 1 tumors, 32 had moderately differentiated, grade 2 tumors, and nine had poorly differentiated, grade 3 tumors. For each category, the proportion of dogs free of disease is plotted against time (in days).[41]

immune-mediated, or allergic disease. It is variably responsive to combination chemotherapy and has a good prognosis if responses are noted. Lymphomatoid granulomatosis must be recognized as a distinct disease entity that has a better prognosis than other, more commonly diagnosed primary or metastatic canine lung diseases.[15,54]

Malignant histiocytosis
Malignant histiocytosis originally was reported in 10 male and one female Bernese mountain dogs.[56]

Nine of the dogs were closely related, which suggests a genetic predisposition. However, other breeds also are at risk, including the rottweiler and golden retriever.[57-60] Most patients are middle aged (range, 4 to 10 years; mean, 7 years). Respiratory signs are the most common complaint, although weight loss, lethargy, and anorexia also may be noted. Most patients have radiographic evidence of a large pulmonary nodule (larger than 5 cm in diameter), and smaller nodules also are visible. Clinically or at necropsy, all patients have metastasis beyond the lung tissue. The most common

A   B
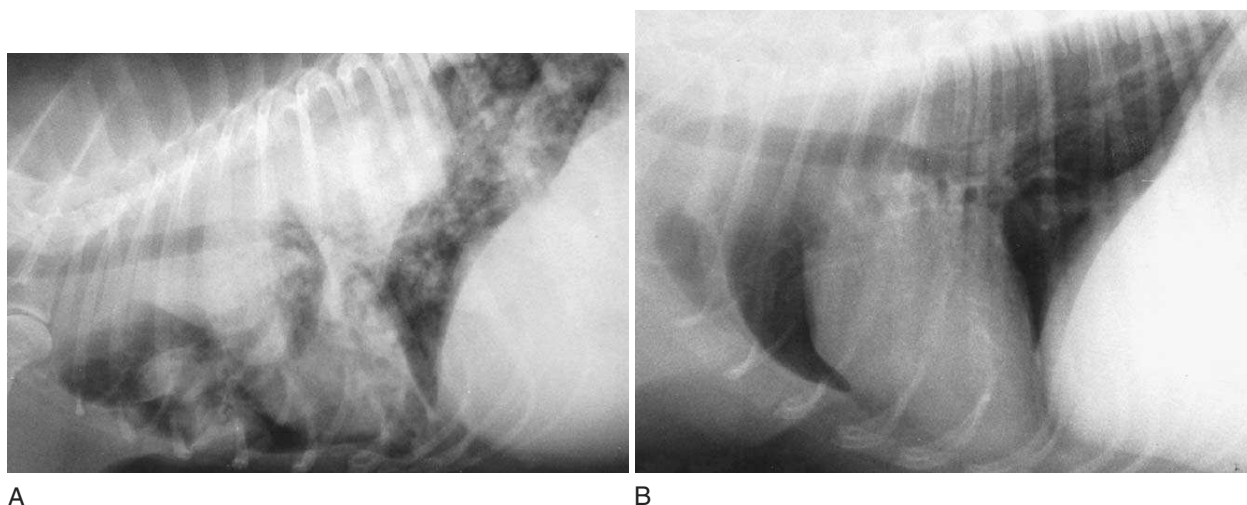
Figure 22-12

A, Lateral radiograph of a 3-year-old dog demonstrating a large perihilar mass with additional peripheral parenchymal lung pathology (confirmed by histology as PLG). **B,** Lateral radiograph of the dog in (**A**) 1 year after chemotherapy with cyclophosphamide, vincristine, and prednisone. Chemotherapy was given for 6 months, and the dog remains in remission 2 years later.

sites of metastasis are the lymph node, liver, kidney, and central nervous system. The histologic diagnosis is not always straightforward and may require special stains for confirmation.[60,61] (A complete discussion of the treatment and prognosis for malignant histiocytosis is presented in Chapter 32, section F.)

Lung cancer in the cat

Most of this chapter has dealt with canine lung cancer, but the general principles also apply to the cat. Cats are much less prone to developing primary lung cancer than are dogs; metastatic disease of the lung is far more common than primary lung cancer in this species. The general distribution of histologic types is similar in the dog and the cat.[10,62,63] In a recent report on the survival of cats after surgery for primary lung tumors, only the histologic grade was predictive of outcome; patients with poorly differentiated tumors had a median survival time of 2.5 months, and those with moderately differentiated tumors had a median survival of 23 months.[63]

An unusual syndrome seen in the cat is primary carcinoma of the lung (usually squamous cell carcinoma) with metastasis to multiple digits and other sites.[64-66] Most cats have swelling of several toes and concomitant radiographic evidence of a lung mass. The lung mass (often solitary) is presumed to have metastasized to the digits. Why the digit is a suitable "soil" for metastasis is unclear. Treatment has been unrewarding to date.

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SECTION D
Canine Nasosinal Tumors

Michelle M. Turek and Susan E. Lana

INCIDENCE AND RISK

Tumors of the nasal cavity and paranasal sinuses account for approximately 1% of all neoplasms in dogs.[1] The average age of dogs with this disease is approximately 10 years, although canine patients as young as 1 year have been reported.[2] Medium and large breeds may be more commonly affected.[2] A slight male predilection has been suggested.[2,3] It has been speculated, but is unproven, that dolichocephalic (long nosed) breeds or dogs living in urban environments, with resultant nasal filtering of pollutants, may be at higher risk.[3-5] Exposure to environmental tobacco smoke was associated with an increased risk of nasal cancer in a group of dogs in one study,[6] but the same was not true in a subsequent study.[5] Indoor exposure to fossil fuel combustion products, such as those produced by coal or kerosene heaters, may contribute to the suggested environmental component of this cancer.[5]

PATHOLOGY AND NATURAL BEHAVIOR

Carcinomas, including adenocarcinoma, squamous cell carcinoma, and undifferentiated carcinoma, represent nearly two thirds of canine intranasal tumors.[7] Sarcomas (usually fibrosarcoma, chondrosarcoma, osteosarcoma, and undifferentiated sarcoma) comprise the bulk of the remaining cancers.[8] Both carcinomas and sarcomas are characterized by progressive local invasion. The metastatic rate generally is considered low at the time of diagnosis but may be as high as 40% to 50% at the time of death, which usually is attributable to the primary disease rather than metastatic lesions.[2] The most common sites of metastasis are the regional lymph nodes and the lungs.[2,8] Less common sites include the bones, kidneys, liver, skin, and brain.[9-11]

Rare tumors of the nasosinal region in dogs include round cell tumors, including lymphoma (unlike in cats, in which this is a common nasal tumor), mast cell tumors, and transmissible venereal cancer. Other malignancies include hemangiosarcoma, neuroendocrine carcinoma, nerve sheath tumor, fibrous histiocytoma, rhabdomyosarcoma, and leiomyosarcoma.[2,12-19] The biologic behavior of these less common malignancies is not well defined. Benign lesions, such as polyps and fibromas, also may be seen.

Recently, a number of immunohistochemical studies have attempted to elucidate possible molecular mechanisms associated with canine nasosinal tumorigenesis. In one study using a single polyclonal antihuman antibody, nuclear p53 accumulation was detected in nearly 60% of nasal adenocarcinomas (11 of 19), which suggests that overexpression of a mutated p53 tumor suppressor protein may play a role.[20] Cyclooxygenase-2 expression has been detected to varying degrees in most nasosinal epithelial tumors sampled,[21,22] and in normal paratumoral respiratory epithelium and stromal tissue.[21]

HISTORY AND CLINICAL SIGNS

Although many intranasal diseases have overlapping clinical signs, a strong presumption of cancer should be made in older animals with an intermittent and progressive history of unilateral (initially) epistaxis or mucopurulent discharge (or both). The average duration of clinical signs before diagnosis is 3 months[1];

A

B
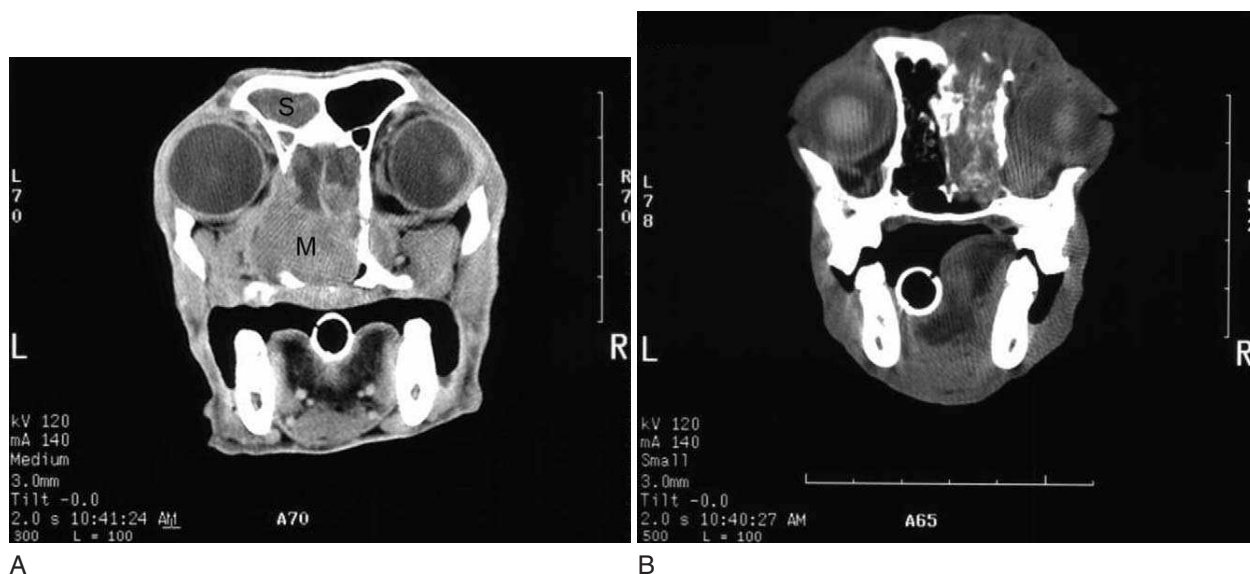
Figure 22-13

A, Contrast-enhanced, computed tomography (CT) image of a dog with nasosinal cancer taken in the transverse plane at the level of the orbit and the olfactory bulb of the brain. Note the contrast-enhancing mass *(M)* in the nasopharynx, which is causing erosion of the frontal and palatine bones. The tumor has invaded the left retro-orbital space and the cranial vault, resulting in deviation of the falx cerebri. Non-contrast-enhancing material in the left frontal sinus *(S)* suggests accumulation of nasal exudate. **B,** Non-contrast-enhanced CT image of a dog with nasosinal cancer taken in the transverse plane at the level of the orbit. Note the soft tissue attenuating mass in the right nasal cavity. Erosion of the frontal bone has allowed the tumor to extend into the subcutaneous tissues on the dorsum of the head; erosion of the palatine bone has allowed invasion of the right retro-orbital space.

these most commonly include epistaxis, bloody or mucopurulent nasal discharge, facial deformity from bone erosion and subcutaneous extension of the tumor, sneezing, dyspnea or stertorous breathing, exophthalmus, and ocular discharge as a result of mechanical obstruction of the nasolacrimal duct.[2,7] Differential diagnoses for animals with these clinical signs include fungal (*Aspergillus* sp.) or bacterial rhinitis, lymphocytic-plasmacytic rhinitis, bleeding disorders, hypertension, foreign body, trauma, and developmental anomalies (e.g., cystic Rathke's clefts).[23-25] If facial deformity is present, the diagnosis almost always is cancer; however, aspergillosis, sporotrichosis, and a rare, benign condition, angiomatous proliferation of the nasal cavity, also can cause facial deformity.

Clinical signs can be alleviated by a variety of symptomatic treatments, including antibiotics, steroids, and nonsteroidal antiinflammatory drugs. An initial response to these treatments should not diminish the diagnostician's index of suspicion for neoplasia in older dogs with clinical signs consistent with cancer.

On rare occasions, animals with tumors involving the caudal region of the nasal cavity may have only neurologic signs (e.g., seizures, behavior change, paresis, circling, and obtundation) caused by direct invasion of the cranial vault.[26] However, the absence of neurologic signs does not rule out tumor extension into the cranial vault, because most dogs with nasal tumors that extend into brain do not have related clinical signs.

DIAGNOSIS AND STAGING

A definitive diagnosis of nasosinal cancer requires a tissue biopsy, even though diagnostic imaging and historical information can be highly suggestive. Coagulation disorders must be ruled out before biopsy, because some bleeding during the procedure is to be expected. Attention should be paid to the platelet count, appropriate clotting at venipuncture sites, and the presence of hematuria, retinal hemorrhage, or petechial hemorrhages. A clotting profile (prothrombin time [PT], activated partial thromboplastin time [APTT]), bleeding time, or activated clotting time (ACT) also can be done.

The superior imaging value of CT over conventional radiographs for canine nasal disease, including neoplasia, is well documented.[23,27-29] CT provides improved anatomic detail, which allows accurate determination of the extent of the tumor (staging) and localization of nasal cavity abnormalities (Figure 22-13).[23,27-30] It also facilitates evaluation of the integrity of the cribriform plate and identification of possible tumor extension

into the cranial vault. In addition, CT imaging is useful for computer planning of radiation treatments to ensure optimal radiation dose delivery.

Certain CT findings have been correlated with a diagnosis of cancer in dogs with nasal disease.[23,30] None of these, alone or in combination, is definitive for neoplasia. [23,30] These findings include destruction of the ethmoid bones, destruction of the bones surrounding the nasal cavities, abnormal soft tissue in the retrobulbar space, hyperostosis of the lateral maxilla, and patchy areas of increased density within an abnormal soft tissue opacity.[30] Based on a recent report, CT appears to be more accurate than radiography at presumptively differentiating causes of chronic nasal disease, specifically differentiating between neoplasia and fungal rhinitis, the most common causes of chronic nasal disease in dogs.[23]

Conventional radiography can still have a place in the diagnostic workup of dogs suspected of having nasal tumors. Despite the inherent limitation of tissue superimposition, the sensitivity of radiography in detecting major nasal cavity abnormalities in dogs with nasal tumors is comparable to that of CT when tumors are sufficiently advanced to cause clinical signs.[27,31] Certain radiographic signs have been correlated with a positive predictive value for neoplasia.[32] These include the presence of a soft tissue opacity, loss of turbinate detail affecting the entire ipsilateral nasal cavity, invasion of bones surrounding the nasal cavity, and soft tissue/fluid opacities within the ipsilateral frontal sinus (Figure 22-14).[32] As is true for CT changes, no radiographic sign is definitively diagnostic for neoplasia.[32] Nasal radiography, therefore, represents a readily accessible and less costly screening procedure that can guide further diagnostics, including biopsy, additional imaging, or both.[33] Standard radiographs taken under anesthesia include lateral, dorsoventral (DV), frontal sinus, and open-mouth oblique views. The most informative views are the open-mouth DV oblique view (to show the caudal nasal cavity and cribriform plate) and the isolated nasal cavity exposure with the film placed in the mouth and exposed in the DV plane.

A tissue biopsy should be obtained while the patient is still under anesthesia for diagnostic imaging. Suitable samples can be acquired by a variety of techniques.[34,35] These include vigorous nasal flushing to dislodge pieces of mass lesions; transnostril blind biopsy using cup forceps or a bone curette; fiberoptic-guided biopsy; fine-needle aspiration or punch biopsy of facial deformities; rhinotomy; and transnostril aspiration and core biopsy (Figure 22-15). Transnostril aspiration and core biopsy involves passing either a punch biopsy needle or a large-bore (3 to 5 mm) plastic cannula into the nasal cavity through the nostril and directing it to the tumor (Figure 22-16).[34] With any transnostril technique, it is important to avoid
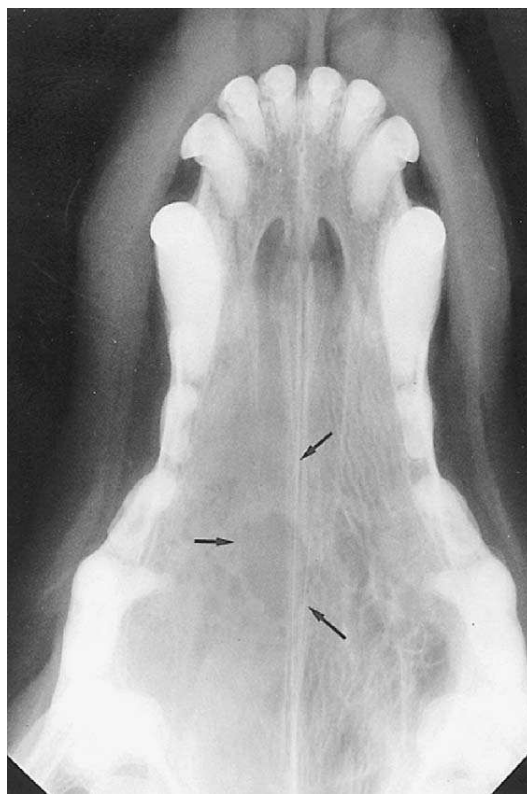
Figure 22-14

Dorsoventral radiograph of the nasal cavity for which the film is placed in the mouth. Note the asymmetry from side to side. Bone lysis *(small arrows)* and loss of turbinate detail imply the presence of a tumor in the caudal half of the nasal cavity.

penetrating the cribriform plate. The biopsy instrument should be marked with tape or cut off at a length that ensures that the instrument does not penetrate farther than the distance from the tip of the naris to the medial canthus of the eye (Figure 22-17). Tumors usually are strongly suspected when white or yellow tissue is obtained rather than turbinate or mucus only. Mild to moderate hemorrhage is to be expected and subsides within several minutes. It is important to ensure the integrity and inflation of the endotracheal tube cuff during any of these procedures. If hemorrhage is severe, the unilateral carotid artery can be permanently ligated, but this is rarely indicated.[36]

Attempts at nasal washing and fluid retrieval for cytologic examination generally have been unrewarding and are not recommended as the sole means of diagnosis.[1] Brush cytology also has been described, but it often is not diagnostic.[37] Rhinoscopy can be used to visualize the nasal cavity, if necessary, and to guide biopsy, although samples collected in this manner often are small and superficial.[38]

A

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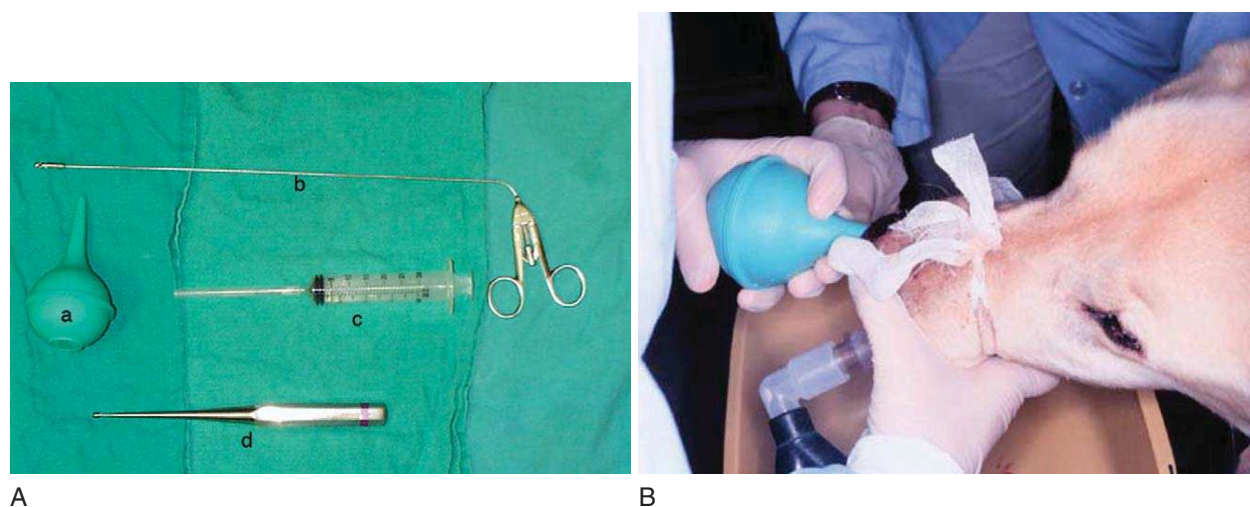
Figure 22-15

A, Several techniques can be used to procure tissue biopsy material from dogs with nasal tumors. A bulb syringe *(a)* can be used to flush out tumor material, or a biopsy forceps, plastic cannula, or bone curette can be inserted through the nostril. **B,** To flush biopsy tissue in an anesthetized dog with an endotracheal tube, the contralateral nasal passage is occluded and flushing pressure is created with a saline-filled bulb syringe; the tissue is flushed back through the nasopharynx and out the mouth into a collection bowl.

Three staging systems for canine nasosinal neoplasia have been proposed on the basis of local tumor extent and bony erosion (Box 22-1).[3,39,40] The prognostic significance of the local tumor stage remains controversial.

Regional lymph node cytology is positive for metastasis at diagnosis in as many as 10% of cases and is most commonly associated with carcinoma. Enlarged regional (mandibular, superficial cervical) lymph nodes should be sampled for cytology to differentiate between a reactive process and metastasis. Thoracic radiographs usually are negative for metastasis at initial presentation.[1,2]

Hematologic and biochemical findings generally are not contributory in dogs with nasosinal tumors. In rare cases, paraneoplastic erythrocytosis and hypercalcemia have been documented.[41-43]

If central nervous system signs exist and advanced imaging is not possible, a cerebrospinal fluid (CSF) sample can be procured to help determine potential extension of disease to the dura or farther across the cribriform plate.[26] Increased CSF pressure and protein and, rarely, increased cell counts are suggestive of brain involvement.[44]

TREATMENT AND PROGNOSIS

Therapy is directed primarily at control of local disease, which usually manifests in a relatively advanced stage in a critical location near the brain and eyes (Figure 22-18). Without treatment, the median survival time of dogs with nasal carcinoma is 95 days (95% confidence interval,

73 to 113 days) as reported in a retrospective case series of 139 dogs.[44a] Prognosis of dogs with epistaxis appears worse than in dogs without epistaxis (median survival time 88 days versus 224 days).[44a] Bone invasion occurs early, with nasosinal neoplasia, and curative surgery is virtually impossible. Although surgical removal by means of rhinotomy has been recommended, a high rate of acute and chronic morbidity, without significant extension of life, greatly limits the utility and indication for this procedure as a sole form of treatment.[1,7,45-47] The median survival time after surgery alone is approximately 3 to 6 months, similar to that reported for no treatment.[1,45-47] In one study, surgery in conjunction with low-energy orthovoltage irradiation provided the best clinical outcome, with respect to median survival time, compared to other treatment modalities[48]; however, more recent reports have not supported this finding (Table 22-1).[11,49]

Radiation therapy using high-energy megavoltage (MeV) equipment (cobalt source or linear accelerator) as the sole treatment modality has become the treatment of choice for canine nasosinal tumors (see Table 22-1). It has the advantage of treating the entire nasal cavity, including bone, and its use has been associated with the greatest improvement in survival. Although surgical removal of the tumor before MeV radiation has not been shown to improve clinical outcome,[1,47,48,50] controlled studies have not been done for this combination. The median survival time after MeV radiation alone with curative intent (i.e., full course or definitive radiation) ranges from 8 to 19.7 months.[39,40,48,51-54] The 1- and 2-year survival rates range from 43% to 60%
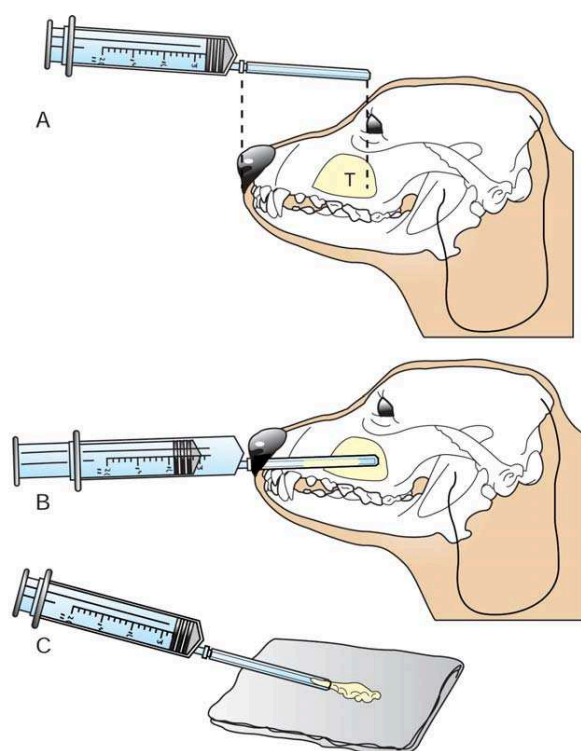
Figure 22-16

A, A caudally situated tumor *(T)* in a dog. To avoid injury to the brain, the plastic cannula for the core aspirate has been shortened such that it extends no deeper than the medial canthus of the eye. **B,** The cannula is introduced into the nasal cavity through the nares. Slight resistance usually is felt as the tumor is entered. Negative pressure is applied as the cannula is redirected at various angles. **C,** Tissue and blood are expelled onto a gauze sponge, where blood is separated from tissue. The tissue is submitted for histologic evaluation.

Figure 22-17

Regardless of the biopsy technique chosen, any instrument that is passed through the naris must be measured; it also should be marked with tape or cut off at a length that ensures that the instrument does not penetrate farther than the distance from the tip of the naris to the medial canthus of the eye. This ensures that the instrument does not pass through a potentially compromised cribriform plate.

(1 year) and 11% to 44% (2 years).[39,40,51,53,54] Doses of 40 to 54 Gray (Gy) usually are delivered in 10 to 18 treatments of 3 to 4.2 Gy fractions over 2 to 4 weeks to the entire nasal cavity and frontal sinuses (if indicated after imaging).[39,40,48,51-54] True statistical comparisons between reports are not possible because of inconsistencies in methodology (even within individual reports) with respect to total dose, fraction number, dose per fraction, treatment schedules, use of CT staging, use of computerized treatment planning, response monitoring, and statistical assessment. In addition, differences in tumor type and tumor stage affect patient outcome. Computerized treatment planning using CT images of the patient in treatment position can greatly enhance normal tissue sparing while ensuring appropriate dose distribution within the tumor. The median survival time ranges from 11 to 19.7 months in dogs treated with CT-staged, computer-planned, MeV irradiation to a minimum of 41 Gy.[39,51-54]

Radiation therapy can induce normal tissue complications in the radiation treatment field. In general, acute and late toxicities affect rapidly and slowly renewing tissues, respectively, depending on the daily dose, total dose, overall treatment time, and volume of tissue treated.[55] For these reasons, the severity of side effects can vary among protocols, depending on the tissues included in the radiation field. Acute toxicities associated with irradiation of nasosinal tumors typically involve the oral cavity (oral mucositis), nasal cavity (rhinitis), skin (desquamation), and eye (keratoconjunctivitis and blepharitis) (Figure 22-19).[39,40,51,56] Acute effects develop and progress during the course of treatment and resolve within about 2 to 8 weeks after therapy.[39,40,51,56] Oral antibiotics, pain medication, and/or artificial tears may be needed to support the patient until the effects resolve. If oral mucositis is severe, resulting in inadequate nutritional intake, esophagostomy or gastrostomy tube feedings may be necessary in the short term.

Late radiation effects, although less common than acute effects, are ultimately more detrimental and long lasting. Their development should be prevented, if possible, with appropriate treatment planning. In late effects, slowly proliferating tissues are affected, including the ocular lens (cataracts), cornea (keratitis, atrophy, keratoconjunctivitis

Box 22-1	Staging Systems for Canine Nasosinal Tumors

World health organization clinical stages (TNM)[2]

T—Primary tumor

T_0 No evidence of tumor
T_1 Tumor ipsilateral, minimal or no bone destruction
T_2 Tumor bilateral and/or moderate bone destruction
T_3 Tumor invading neighboring tissues

N—Regional lymph nodes (RLN)

N_0 No evidence of RLN involvement
N_1 Movable ipsilateral nodes
N_2 Movable contralateral or bilateral nodes
N_3 Fixed nodes

M—Distant metastasis

M_0 No evidence of distant metastasis
M_1 Distant metastasis detected (including distant nodes)

Théon modified staging system[40]

Stage 1   Unilateral or bilateral neoplasm confined to the nasal passage without extension into the frontal sinuses

Stage 2   Bilateral neoplasm extending into the frontal sinuses with erosion of any bone of the nasal passage

Adams modified staging system[39]

T1   Confined to one nasal passage, paranasal sinus, or fontal sinus with no bony involvement
T2   Any bony involvement, but no evidence of orbital, subcutaneous, or submucosal mass
T3   Involvement of orbit or subcutaneous or submucosal mass
T4   Tumor extension into nasopharynx or cribriform plate

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From Patnaik AK: Canine sinonasal neoplasms: clinicopathological study of 285 cases, J Am Anim Hosp Assoc 25:103-114, 1989; Théon AP, Madewell BR, Harb MF et al: Megavoltage irradiation of neoplasms of the nasal and paranasal cavities in 77 dogs, J Am Vet Med Assoc 202:1469-1475, 1993; and Adams WM, Miller PE, Vail DM et al: An accelerated technique for irradiation of malignant canine nasal and paranasal sinus tumors, Vet Radiol Ultrasound 39:475-481, 1998.

Figure 22-18

Cross section of a dog's skull with a typical intranasal carcinoma. Note the middle to caudal position of the tumor in the nasal cavity, the erosion of the dorsal nasal bones, the dark mucus in the frontal sinus secondary to obstruction, and the proximity of the tumor to the cribriform plate. Complete surgical resection is impossible.

sicca), anterior uvea (uveitis), retina (hemorrhage and degeneration), neuronal tissue (brain necrosis, causing neurologic changes and/or seizures and optic nerve degeneration), bone (osteonecrosis), and skin (fibrosis) (Figure 22-20).[39,40,51,56-59] Late complications develop months to years after therapy and generally are irreversible.

Overall, although most dogs with nasosinal tumors can enjoy long periods of tumor control after radiation therapy, the long-term prognosis is poor (see Table 22-1). Most dogs, even those treated with definitive radiotherapy, die or are euthanized as a result of local disease progression. An investigation of treatment failure patterns after full course MeV irradiation showed that the median duration of local control in 24 dogs was 312 days.[60] Marked tumor regression (90% reduction in size) was observed using CT in 46% of cases and was associated with a longer duration of local control than that seen in dogs in which tumor response to radiation was less favorable (389 versus 161 days).[60] Most of the dogs in that series experienced local progressive disease,[60] which affirms the need for more effective treatment than that afforded by standard veterinary radiation regimens.

Various approaches to improve local control have been investigated.

1. A recent report on full course preoperative radiotherapy *followed* by surgical exenteration of residual or recurrent disease in a small series of dogs (n = 13) showed promise, with a median survival time of 47 months compared to 19 months for dogs treated with radiation alone (see Table 22-1).[53] The combination treatment was associated with an increased incidence of late effects, including rhinitis (bacterial and fungal), osteomyelitis, and fistula formation.[53] A larger group of dogs must be treated in this manner to confirm these findings.

TABLE 22-1 Summary of Selected Articles on Treatment of Nasosinal Tumors in Dogs with Radiation

	Adams et al.[48]	Northrup et al.[11]	Théon et al.[40]	McEntee et al.[51]	Adams et al.[39]	Lane et al.[54]	Adams et al.[53]	Adams et al.[53] (historic controls)	Mellanby et al.[70]
Number of dogs	67	42	77	27	21	51	13	40	56
Adjuvant treatment									
Surgery	41/67	42/42	21/77*	6/27	None	None	11/13 after RT	None	None
Chemotherapy	None	None	None	None	14/21†	51/51‡	None	None	None
Survival									
Median (months)	8.1	7.4	12.6	12.8	14.3	15.8	47.7	19.7	7
1 year/2 year (%)	38/30	37/17	60/25	59/22	60/36	Not reported	76/69	68/44	45/15
Dose (Gy)									
Mean	43								
Median	48	48	48	41-54	42	54	42	42	36
Number of fractions	5-10	12	12	10-12	9-10	18	10	10	4
Radiation source	Variable	Orthovoltage (low energy)	Cobalt	Cobalt	Cobalt	6 MeV linear accelerator	Cobalt	Cobalt	4 MeV linear accelerator
Prognostic indicators/conclusions	SX + low energy; no advantage to SX + high energy	SX + low energy less effective than reported by Adams[38]; resolution of clinical signs favorable for survival; facial deformity not favorable for survival	SX did not affect outcome; SA > CA	SX did not affect outcome; SA = CA	Immuno-modulator did not affect outcome	Combination was well tolerated	Post-SX complications include rhinitis (bacterial or fungal), osteomyelitis, fistula.		Palliative protocol; resolution of clinical signs in 95% of dogs

*Measurable disease remained in all dogs after the surgical procedure.
†Immunotherapy was given using liposome-encapsulated MTP-PE.
‡A slow-release formulation of cisplatin was given as chemotherapy.
SX, Surgery; SA, sarcoma; CA, carcinoma; RT, radiation.

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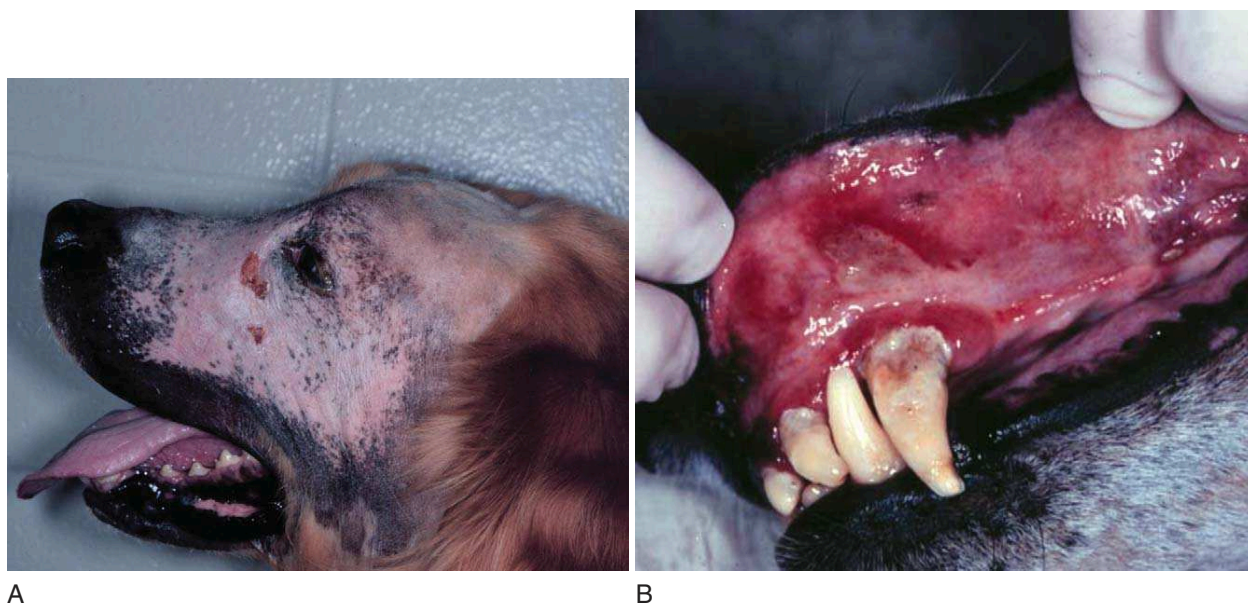
Figure 22-19

Potential acute effects of radiation therapy for intranasal tumors. **A,** A severe example of desquamation after radiation therapy in a dog. **B,** Oral mucositis in a dog after radiation therapy.

2. A logical and intuitive approach to improve local control is to increase the radiation dose. This has been investigated using a boost technique in one study in which the total radiation dose was escalated to 57 Gy without an increase in overall treatment time. The treatment proved too toxic with respect to acute effects and resulted in radiation-related deaths in one third of the dogs evaluated.[56] It appears that normal tissue tolerance does not allow dose escalation within standard overall treatment times using currently available radiation planning and delivery techniques.

3. The use of radiosensitizers in conjunction with ionizing radiation has been reported. A recent Veterinary Radiation Therapy Oncology Group (VRTOG) pilot study described the use of gemcitabine as a radiosensitizer for nasosinal carcinoma.[61] Gemcitabine was given intravenously at a dosage of 50 mg/m^2 twice weekly before daily radiation therapy. The authors reported significant hematologic toxicity (neutropenia) and local acute tissue complications associated with this dose and schedule. In another recent report, low-dose cisplatin (7.5 mg/m^2 given intravenously every other day) administered in conjunction with full course radiotherapy was well tolerated and did not appear to cause an increase in local acute or late radiation effects.[52] The efficacy of this approach with respect to improvement in clinical outcome is not known. A combination of radiation and cisplatin, administered intramuscularly throughout therapy using a slow-release polymer system (open-cell polylactic acid polymer impregnated with platinum [OPLA-Pt]), has been shown to be well tolerated; survival times were similar to those in other studies that used radiation therapy alone (median survival time, 474 days).[54]

4. In radiotherapy the goal of treatment is to deliver the maximum radiation dose to the tumor while confining the dose to surrounding normal tissues to an amount below their tolerance level.[62] Currently, in veterinary medicine, maximally optimized radiation delivery is achieved through the use of conventional computerized treatment planning that allows the use of multiple treatment fields directed at the patient from different angles, differential weighting of beams, and beam shape modulators, such as lead blocks and wedges, to tailor the distribution of radiation dose to each patient as much as possible. Even with the development of more sophisticated planning systems (i.e., three dimensional versus two dimensional treatment planning), these conventional methods have limitations that prevent escalation of the radiation dose without also increasing complications.[56]

A potential solution to the problem is to deliver multiple radiation fields with nonuniform beam intensity when the goal is to spread the dose around, so that larger volumes of normal
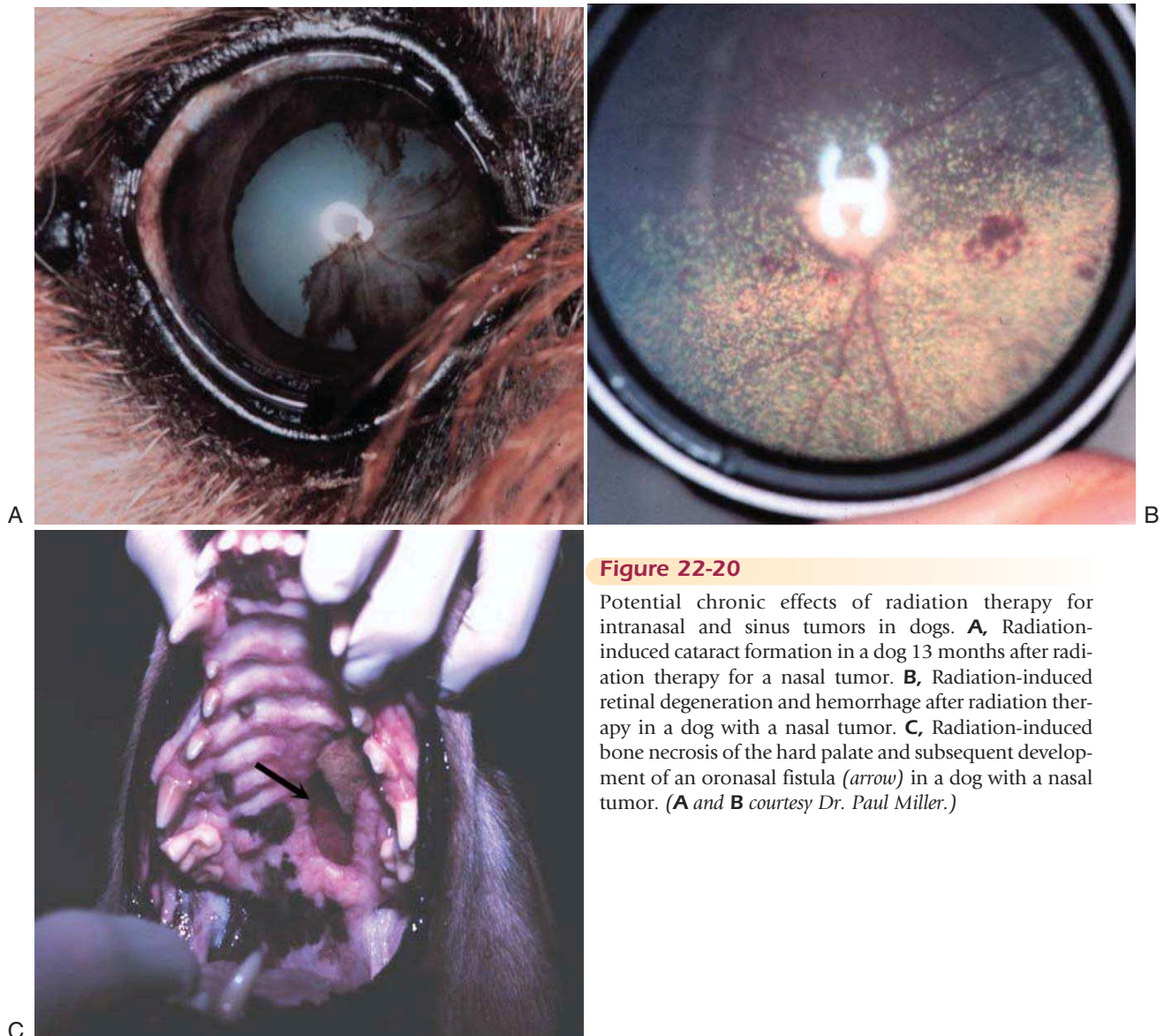
Figure 22-20

Potential chronic effects of radiation therapy for intranasal and sinus tumors in dogs. **A,** Radiation-induced cataract formation in a dog 13 months after radiation therapy for a nasal tumor. **B,** Radiation-induced retinal degeneration and hemorrhage after radiation therapy in a dog with a nasal tumor. **C,** Radiation-induced bone necrosis of the hard palate and subsequent development of an oronasal fistula *(arrow)* in a dog with a nasal tumor. (**A** *and* **B** *courtesy Dr. Paul Miller.*)

tissue generally are exposed to more modest doses of radiation, but the doses integrate into a higher total dose throughout the volume of the tumor.[63,64] This results in conformal distribution of the radiation dose to the tumor with sparing of sensitive normal tissues.[64] This rapidly emerging technology is called intensity-modulated radiation therapy (IMRT). Unlike conventional treatment-planning systems, in which the operator's goal is to conform the spatial distribution of dose to the tumor while trying to minimize the dose to surrounding normal tissues, IMRT treatment plans are generated by "automated optimization," in which the computer itself determines the optimal nonuniform radiation exposure that must be

delivered to achieve the desired conformal dose pattern.[62,65]

The delivery of IMRT is achieved by using a large number of beams of varying radiation intensity that are modulated spatially and temporally.[62] The variation in beam intensity (modulation) is realized using a multileaf collimator. This is a collimator that is made of multiple leaves that move rapidly and independently under computer control, customizing each beam in shape and intensity to the patient. As a result, IMRT has the potential to achieve a much higher degree of target conformity and normal tissue sparing, especially for tumors with complex shapes, than conventional treatment-planning techniques.[65] Although IMRT has obvious

theoretical benefit over conventional techniques, clinical data on toxicity and therapeutic outcome are needed in both human and veterinary medicine before IMRT can be accepted as the gold standard. A proof-of-principle study showing the spatial accuracy of IMRT delivery has been done in normal dogs.[66]

Helical tomotherapy is a novel form of radiation delivery that integrates IMRT and helical CT.[67] Using an intensity-modulated, fan-shaped radiation beam that rotates around the patient in a helical fashion, like the diagnostic x-ray beam of a helical CT scanner, this form of IMRT delivers conformal radiation "slice by slice" rather than using conventional static fields.[67,68] The CT component of the system, using high-energy x-rays, allows verification of patient positioning before and during treatment, which is critical in the delivery of such conformal therapy.[67] In addition, it allows calculation of the administered radiation dose so that any variations that occur in the patient's treatment can be corrected.[67-68,68b]

These are novel concepts in radiation therapy. They are applicable to cases involving tumors of complex shape that are close to critical normal tissues, which may have a radiation tolerance that limits adequate dose delivery to the tumor, resulting in poor tumor control. The merits of this approach are being prospectively investigated in dogs with nasosinal cancer. Preliminary results suggest that IMRT/helical tomotherapy substantially reduced the dose delivered to the eyes and resulted in bilateral ocular sparing in a small series of dogs.[69] If IMRT techniques are in fact proven to induce less treatment-related toxicity and to be equally as effective therapeutically as conventional techniques, there will be a theoretical opportunity for tumor dose escalation without increasing the number of radiation treatments and the overall treatment time, which should translate into improved tumor control.

5. A coarse fraction, palliative radiotherapy protocol recently was evaluated in 56 dogs with no evidence of metastasis (see Table 22-1).[70] Four treatments of 9 Gy given at 7-day intervals resulted in alleviation of clinical signs in 95% of the dogs by the end of the protocol. The median survival time was 7 months. Tumor responses and the duration of palliation were not evaluated; however, most of the dogs were euthanized because of recurrent clinical signs. Radiation-related side effects included alopecia, erythema, conjunctivitis and mucositis, which required antibiotics in 39% of the animals. Nine dogs also received steroids or nonsteroidal antiinflammatory drugs for acute side effects.

The possible confounding impact of these drugs on clinical improvement must be considered.

6. Cisplatin chemotherapy used alone has been shown to benefit some dogs. A clinical response rate of 27%, including one radiographically confirmed complete remission, was reported in a small series of 11 dogs.[71] All the dogs experienced alleviation of clinical signs, but the median survival time was only 5 months. A recent report by Langova and coworkers[72] evaluated a combination chemotherapy protocol of doxorubicin, carboplatin, and oral piroxicam in eight dogs with advanced nasal tumors. A clinical response rate of 75% was observed, including four complete responses, confirmed by CT imaging. All the dogs experienced resolution of clinical signs after one or two doses, and the protocol was well tolerated. These preliminary results are favorable, but the case number was small, and more dogs need to be treated in this manner to confirm these findings.

Other therapeutic approaches include brachytherapy, immunotherapy, cryotherapy, and photodynamic therapy (PDT). Intracavitary radiation using radioactive isotopes (brachytherapy) has been evaluated after surgical removal of nasosinal tumors in dogs.[73,74] Potential problems associated with this type of radiation include nonhomogeneous dose distribution and radiation exposure to personnel. The question of whether brachytherapy improves survival over traditional external beam radiation has not been answered. Nonspecific immunomodulation (e.g., levamisole, mixed bacterial vaccine) and cryosurgery have not been shown to improve survival times.[1,75] Case reports on PDT recently have been published, but the results are too preliminary to draw conclusions.[76]

When treatments fail to control epistaxis, unilateral or bilateral carotid artery ligation can palliate the symptoms in dogs for up to 3 months or longer without hypoxic damage to the brain.[35]

The importance of prognostic factors in the treatment of canine nasosinal tumors remains controversial. Negative predictors of survival from various studies include age (over 10 years),[77] advanced local tumor stage,[39,40,77] metastatic disease,[47,77] and histologic subtype (carcinoma, particularly squamous cell or undifferentiated).[40,48] However, none of these factors has been consistently validated in other studies, and their true significance is unresolved.

A major pitfall of many veterinary studies with respect to assessment of treatment efficacy in canine nasosinal cancer is the evaluable endpoint. Tumor response and the time to tumor progression are the most representative measures of treatment efficacy. Regular diagnostic imaging, ideally with CT or MRI, would be required to allow determination of these endpoints. Unfortunately,

because of the high cost and the need for anesthesia, follow-up is rarely done in this manner. Furthermore, a problem exists in analyzing the return of clinical signs as tumor recurrence, because similar signs can result from treatment (radiation, surgery, or both).[78] Assessment of the survival time, common in most veterinary nasosinal cancer studies, is biased by the use of additional treatments upon suspicion of progressive disease and by the decision for euthanasia, which can vary greatly from one pet owner to another.

FELINE NASOSINAL TUMORS

Nasosinal cancer is less common in the cat than the dog.[79] Although the mean age of affected animals is approximately 10 years,[80,81] feline patients as young as 3 years have been reported.[79] In general, tumors are locally invasive and associated with a low metastatic rate at the time of diagnosis.[81,82]

Clinical signs related to nasosinal tumors overlap with those of other causes of chronic nasal disease.[80] These include nasal discharge, upper respiratory tract dyspnea, sneezing, epistaxis, facial swelling, ocular discharge, lethargy, regional lymphadenopathy, and weight loss.[79-81] Although each of these signs can occur with both neoplasia and rhinitis, it appears that animals with dyspnea, unilateral nasal discharge, and/or bloody nasal discharge are more likely to have neoplasia than rhinitis.[80] The median duration of clinical signs before diagnosis is 2 months.[79,80] Many cats experience temporary alleviation of clinical signs with use of antibiotics or glucocorticosteroids.[79,80] Differential diagnoses for chronic nasal signs include chronic rhinitis, infectious rhinitis, foreign body, nasal polyp, nasopharyngeal stenosis, and trauma.

Lymphoma (most tumors are feline leukemia virus [FeLV] antigen negative) and epithelial neoplasms (carcinoma, adenocarcinoma, squamous cell carcinoma) are the most common tumors of the feline nasal cavity and sinuses. Less frequently reported tumor types include sarcomas (fibrosarcoma, osteosarcoma, chondrosarcoma), mast cell tumor, melanoma, plasmacytoma, olfactory neuroblastoma, and benign lesions such as hemangioma, chondroma, and neurofibroma.[79-81,83]

Diagnostic principles in the cat are similar to those in the dog. A tissue sample is required to make a definitive diagnosis of cancer (Figure 22-21). Both chronic rhinitis and neoplasia have a high prevalence of aggressive radiographic lesions, including erosion, deviation, and proliferation of bone, and differentiating the two can be difficult.[84,85] Although no radiographic sign is entirely specific for neoplasia, unilateral changes, including turbinate destruction, loss of teeth, and lateral bone erosion, as well as displacement of

midline structures, appear to be more prevalent in cats with cancer than in those with chronic rhinitis.[84,85] Similarly, a recent evaluation of CT imaging in 62 cats with nasosinal disease showed that although certain findings may suggest a CT diagnosis of neoplasia over rhinitis (e.g., osteolysis of paranasal bones, extension of disease into the orbit of facial soft tissues, presence of a space-occupying mass, and turbinate destruction), nasal biopsy is necessary for confirmation.[86] As in the dog, this study also showed that, although CT is not more sensitive than conventional radiography at detecting nasal cavity abnormalities, it is more sensitive at localizing these changes and determining the extent of disease.[80]

Even though the metastatic rate of feline nasosinal cancer at the time of diagnosis is reportedly low,[81] any enlarged regional lymph nodes should be evaluated cytologically to differentiate a reactive process from metastasis. In a recent report of 123 cases of feline
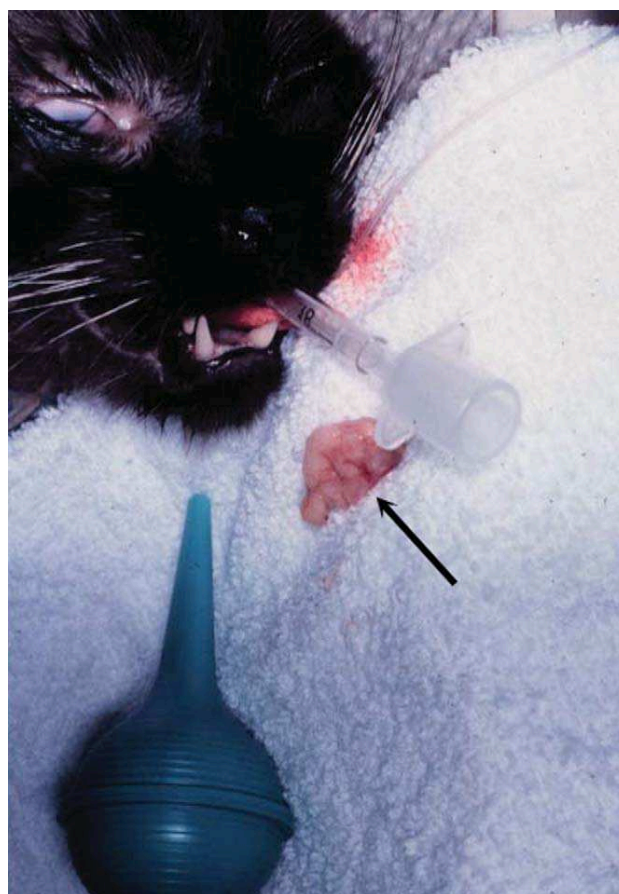
Figure 22-21

A large biopsy tissue sample is procured from a cat with nasal lymphoma *(arrow)* using the bulb syringe flush technique. This technique can also be therapeutic, because it often debulks the nasal passage and increases airflow.

nasosinal cancer, 21 cats had regional lymphadenopathy.[81] None showed cytologic evidence of metastasis. Thoracic radiographs should be evaluated for gross pulmonary metastasis.

Reports of treatment for feline nasosinal tumors are few, and case numbers are small.[82,87-89] In the largest published study, 16 cats with nonlymphoproliferative neoplasms were treated using a definitive course of radiation to a total dose of 48 Gy, resulting in minimal acute radiation toxicity and modest chronic ocular side effects in three cats.[82] The therapy was well tolerated, and the median survival time was 12 months, with 44% of the cats alive at 1 year and 16% alive at 2 years (results very similar to those in the dog). Objective tumor responses were observed. Another report of nonlymphoproliferative tumors treated with a coarse fractionation regimen showed palliation of clinical signs in about half of the animals within 3 months after therapy.[87] The protocol was well tolerated, and the median survival time was 12 months. A larger group of cats must be treated to confirm the utility of this protocol. Reported cases of nasosinal lymphoma treated with radiation or chemotherapy are few, but survival may be favorable.[88-94]

COMPARATIVE ASPECTS[95]

In humans, cancer of the nasal cavity and paranasal sinuses is classified with other cancers of the upper aerodigestive tract in the all-encompassing category of tumors of the head and neck. Tumors of four anatomically defined regions are included: nasal cavity and paranasal sinuses, nasopharynx, oral cavity, and oropharynx. Most tumors that affect these sites are squamous cell carcinomas. However, a large variety of histologically distinct cancers can develop in the nasal cavity and paranasal sinuses, including adenocarcinoma, esthesioneuroblastoma, lymphoma, melanoma, angiosarcoma, and bone or cartilage tumors.

Tumors specifically of the nasal cavity and paranasal sinuses are rare in humans, accounting for the smallest proportion of head and neck cancers. They generally affect individuals over 60 years of age and are twice as common in men as in women. Paranasal sinus cancer is more frequently observed in Japan and South Africa than in other parts of the world.

The etiologic factors for the disease are predominantly environmental. They include exposure to nickel; exposure to the production of chromium, mustard gas, isopropyl alcohol, and radium; and occupations associated with the furniture, textile, and shoe industries.

Tumors at this site are locally invasive, and they typically are advanced at the time of diagnosis. Metastasis occurs relatively late in the course of the disease.

The likelihood of lymph node metastasis at the time of presentation is related to the location of the primary tumor and the tumor stage.

Surgery is the mainstay of treatment. Overall, the survival rate at 5 years is approximately 60% for nasal cavity tumors and 30% for paranasal tumors. More favorable prognoses are associated with smaller lesions when complete resection is achievable. The main form of treatment failure is local recurrence arising from incomplete resection of advanced tumors. When adequate margins cannot be obtained, surgery and radiation often are combined. Radiation is rarely used alone unless surgery is not feasible. Preoperative radiation is preferred for patients whose tumors cannot be resected completely, because it is associated with improved survival, despite the higher surgical complication rate compared with the use of radiation postoperatively.

The role of chemotherapy in cancer of the nasal cavity and paranasal sinuses is not clear, because these cases usually are reported in conjunction with other head and neck tumors. Active chemotherapeutic agents include bleomycin, 5-fluorouracil (5-FU), and cisplatin. These can be delivered intravenously or regionally by intra-arterial administration, which increases drug exposure and reduces systemic toxicity. Multimodality protocols, including chemotherapy, radiation, and surgery, are used for advanced cases, and the results compare favorably to those with patients treated with surgery and radiotherapy. In some advanced cases, intra-arterial chemotherapy combined with radiation therapy achieves results similar to those with radiation and surgery, sparing the patient the effects of major surgery.

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Tumors of the Skeletal System

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OSTEOSARCOMA IN DOGS

Incidence and Risk Factors

Osteosarcoma (OS) is the most common primary bone tumor in dogs accounting for up to 85% of malignancies originating in the skeleton.[1-5] Osteosarcoma is estimated to occur in more than 8000 dogs each year in the United States; however, this is probably an underestimation, since not all cases are confirmed nor recorded.[6,7] The demographics of canine OS have been well reported.[1-5,8-24] It is largely a disease of middle-aged to older dogs, with a median age of 7 years. There is a large range in age of onset with a reported case in a 6-month-old pup[25] and a small early peak in age incidence at 18 to 24 months.[11] Primary rib OS tends to occur in younger adult dogs with a mean age of 4.5 to 5.4 years.[26,27] Osteosarcoma is classically a cancer of large and giant breeds. In a review of 1462 cases of canine OS, dogs weighing more than 40 kg accounted for 29% of all cases, and only 5% of their tumors occurred in the axial skeleton. Only 5% of OS occur in dogs weighing less than 15 kg, but 59% of their tumors originated in the axial skeleton.[23] Increasing weight and, more specifically, height appear to be the most predictive factors for the disease in the dog.[28] The breeds most at risk for OS are Saint Bernard, Great Dane, Irish setter, Doberman pinscher, rottweiler, German shepherd, and golden retriever; however, size seems to be a more important predisposing factor than breed.[1,3,5,8,9,11,12,14,20,24,28] A hereditary basis for the formation of OS has been suspected, based primarily on the (large) breed prevalence of the disease as well as the subjective assessment of increased incidence in some related families. Males are reported to be slightly more frequently affected than females (1.1 to 1.5:1)[1,4,8,11,13,15,17-19] with the exception of the Saint Bernard, rottweiler, and Great Dane and for dogs with primary OS of the axial skeleton (except rib and spine) where affected females outnumber males.[1,17,18,26] However, in 1775 cases of canine OS of all sites treated at Colorado State University between 1978 and 2005, the male-to-female ratio was 1:1 (unpublished data, Colorado State University Animal Cancer Center [CSU-ACC] OS Database). Intact males and females were reported to have an increased risk for OS;[28] however, in the rottweiler breed, male and female dogs that underwent gonadectomy before 1 year of age had an approximate one in four lifetime risk for bone sarcoma and were significantly more likely to develop bone sarcoma than dogs that were sexually intact.[29] There was a highly significant inverse dose-response relationship between duration of lifetime gonadal exposure and incidence rate of bone sarcoma independent of adult height or body weight.

Approximately 75% of OS occurs in the appendicular skeleton, with the remainder occurring in the axial skeleton.[1,26] The metaphyseal region of long bones is the most common primary site with front limbs affected twice as often as rear limbs and the distal radius and proximal humerus being the two most common locations.[16] It is extremely rare for OS to be located in bones adjacent to the elbow, although there is one report of 12 cases located at the proximal radius or distal humerus.[30] There was no prognostic difference in these cases as compared to more common appendicular sites. In the rear limbs, tumors are fairly evenly distributed between the distal femur, distal tibia, and proximal tibia, with the proximal femur a slightly less common site.[1] Primary OS distal to the antebrachiocarpal and tarsocrural joints is relatively rare in dogs.[31] In 116 cases of canine primary OS in the axial skeleton, it was reported that 27% were located in the mandible, 22% in the maxilla, 15% in the spine, 14% in the cranium, 10% in ribs, 9% in the nasal cavity or paranasal sinuses, and 6% in the pelvis.[26] Single reports of OS development in the os penis[32] and the patella[33] exist in the dog. Clinically documentable multicentric OS at the time of initial diagnosis occurs in less than 10% of all cases.[34] Osteosarcoma of extraskeletal sites is

rare, but primary OS has been reported in mammary tissue, subcutaneous tissue, spleen, bowel, liver, kidney, testicle, vagina, eye, gastric ligament, synovium, meninges and adrenal gland.[35-40]

Etiology

The etiology of canine OS is generally unknown. Some have speculated a viral cause because OS can occur in litter mates and may be experimentally induced by injecting OS cells into canine fetii.[41] However, an etiologic virus has not been isolated.

Physical factors

A simplistic theory based on circumstantial evidence is that since OS tends to occur in major weight-bearing bones adjacent to late closing physes and heavy dogs are predisposed, multiple minor trauma and subsequent injury to sensitive cells in the physeal region may occur. This may initiate the disease by inducing mitogenic signals increasing the probability for the development of a mutant lineage. One *in vitro* study comparing the incidence of microdamage in cadaver radii of small- and large-breed dogs found no difference between the groups.[41] OS has been associated with metallic implants used for fracture repair, chronic osteomyelitis, and with fractures in which no internal repair was used.[42-46] Osteosarcoma has also been reported at the site of a bone allograft used for fracture repair 5 years previous.[47] Exposure to ionizing radiation can induce osteosarcoma.[48-56] In plutonium exposed people, 29% and 71% of the OS were in the appendicular and axial skeleton, respectively, with the spine having the most tumors (36%). An almost identical distribution of plutonium-induced OS was reported for dogs injected with ^{239}Pu as young adults in experimental studies. This distribution of osteosarcomas is quite different from the distributions of naturally occurring OS for both species and appears to be related to bone volume and turnover. Similar findings were seen for dogs injected with ^{226}R (radium).[50] A distribution favoring bone marrow volume was seen for dogs exposed to strontium-90.[51] OS is a rare, late complication of radiation therapy in people and dogs.[52-56] Three of 87 (3.4%) spontaneous tumor-bearing dogs treated for soft tissue sarcomas developed OS within the field of radiation.[52] In another experimental study, 21% dogs undergoing intraoperative radiation therapy (greater than 25 Gy) followed by external beam radiation to the vertebral column developed osteosarcoma following treatment.[53] Secondary OS developed between 1.7 to 5 years after radiation in that study, and the authors speculated that high dose of radiation per fraction or total dose may predispose to this serious late effect of irradiation. Osteosarcoma was reported in 3% of 57 dogs irradiated for acanthomatous epulis of the oral cavity in another study.[54]

Postirradiation OS in people comprised approximately 2% to 4% of all osteosarcomas reviewed in two large series.[57,58]

Osteosarcomas have been concurrently seen in dogs with bone infarcts, but it is not clear whether there is any causal relationship.[59-61] Bone infarcts are uncommon, are of unknown etiology, and may be identified as incidental findings by radiography. Bone infarcts are probably not associated with tumor emboli and appear to be more common in smaller breeds. A single report exists of OS occurrence secondary to a bone infarct caused by total hip arthroplasty.[62] A case of OS was also reported to be associated with osteochondritis dissecans in the humoral head[63] and another report documents malignant transformation of an aneurysmal bone cyst.[64]

Molecular and genetic factors

There is a growing body of experimental work and clinical data to support molecular and genetic factors by which OS may develop and progress (Table 23-1).[65-67] Osteosarcoma tumorigenesis in humans results if both retinoblastoma susceptibility locus (Rb gene; see Chapter 4) are altered.[68] Investigators concluded that Rb gene alteration is pertinent to the genesis of most human OS cases and some other bone and soft tissue tumors. Additional genetic evidence suggests that another event in the development of OS is the homozygous alteration of another gene, p53.[69-72] Both the Rb gene and p53 have been proposed to act as tumor suppresser genes (Chapter 1, Section A). The p53 suppressor gene appears to be mutated and overexpressed in canine OS and appears more frequently in appendicular than flat bone sites, which may support it as a factor in the more aggressive form of the disease.[73-79] In several studies, p53 mutations were present and often overexpressed in OS tumor samples indicating a potential causal relationship.[74,76,77] In many cases, the defects occurred in similar sites to what is observed in human tumors.[76] In five canine OS cell lines tested, defective p53 was observed, which resulted in inactivation of three Rb family members.[75] Additionally, when canine OS cells are transfected with wild-type p53 they lose much of their proliferative characteristics and proceed toward apoptosis.[78] Many of the aberrations of the p53 gene detected in these tumors are located in the transactivation, DNA binding, and oligomerization domains.[77]

Alterations in several growth factor, cytokine, or hormone signaling systems have also been documented in the pathogenesis of OS. Growth hormone has been shown to be measurable within appendicular OS samples, indicating a potential cause or response relationship.[80] Studies evaluating the role of insulin-like growth factor-1 (IGF-1) and its receptor IGF-1R and hepatocyte growth factor (HGF) and its receptor c-Met in canine OS cell lines and tumor tissues have shown that these factors may contribute to the malignant

TABLE 23-1 Molecular and Genetic Factors Associated with OS in Dogs

Factor	Comments	Reference
p53	Mutated and/or overexpressed in several investigations	73–79
IGF-1/IGF-1R	May contribute to the malignant phenotype	81
HGF/c-Met	May contribute to the malignant phenotype	82–84
*erb*B-2/HER-2	Overexpressed in several canine OS cell lines	85
PTEN	Mutated or downregulated in a high percentage of canine OS cell lines	87
sis/PDGF	Overexpressed in some canine cell lines	88
Matrix metalloproteinases	Overexpressed in canine OS cell lines	92, 93
Ezrin	A membrane-cytoskeleton linker associated with the metastatic phenotype in canine OS	94
COX-2	Expression upregulated in some canine OS; prognostic in some investigations, not in others	95, 96
Angiogenic factors	VEGF measurable in plasma of dogs with OS; angiostatin present in urine of dogs with OS	97
Telomerase reverse transcriptase gene	Upregulated in some canine OS	107

phenotype.[81-84] Ferracini et al. showed an overexpression of the c-Met oncogene in 10 canine OS tissue samples, including a lung metastasis.[84] Overexpression of erbB-2, the protooncogene that encodes growth factor receptor 2 (HER-2), occurs and is thought to be important in tumor transformation, and growth was seen in 86% of canine OS cell lines and 40% of clinical samples in one study.[85] Overexpression of erbB-2 has been related to poor prognosis in some human cancers; however, amplification of HER-2/neu has been shown to be uncommon in human OS.[86] Another important tumor suppressor, PTEN has been shown to be mutated or downregulated in a high percentage of canine OS cell lines and tumors and may also play a role in the pathogenesis of the disease.[87] The sis oncogene was also found to be overexpressed in a canine cell line.[88] This oncogene encodes for platelet derived growth factor (PDGF) and could contribute to OS pathogenesis in some subsets. Overexpression of c-kit, a tyrosine kinase receptor oncogene that appears to regulate cell differentiation, has been associated with a number of tumors including mast cell tumor in dogs.[89] Overexpression of c-kit as well as an allelic imbalance at the locus that contains the c-kit gene has been shown in human pediatric OS and is predictive of outcome.[90] Evaluation of c-kit in canine OS would be a necessary prerequisite to the use of novel KIT inhibitor therapies.[91]

The matrix metalloproteinases (MMPs) are matrix degradative proteins that may be partially responsible for local disease progression and metastasis (Chapter 2). MMP 2 and 9 have been shown to be present in high concentrations in canine OS cell lines.[92] The presence of these matrix degradative proteins may be partially responsible for local disease progression and metastasis.[93] Similarly, investigations of ezrin, a membrane-cytoskeleton linker, has been shown to be associated with the metastatic phenotype in dogs with OS.[94]

A growing body of information is implicating aberrations in cyclooxygenase enzyme systems, important mediators of prostanoids, in tumorigenesis in dogs. Cyclooxygenase-2 (COX-2) expression appears upregulated in canine OS, and expression levels were shown to be prognostic in one study.[95] A contradictory study failed to document overexpression of COX-2 in OS tissues, whereas overexpression was seen in canine epithelial tumors. [96]

Several factors and events in tumor-related angiogenesis (Chapters 2 and 14, Section E) also may be important in OS development and progression. Blood vessel density was shown to be greater for primary OS cases presenting with metastatic disease than those without metastasis, potentially indicating vascularity as an indicator of biologic behavior.[97] A study evaluating the Spälteholz tissue clarification imaging technique found microvascular density in canine OS to be inhomogeneous, and the authors speculated that this may partially explain the lack of response of local tumor to chemotherapy and radiation and the tendency for local progression following these therapies.[98] Vascular endothelial growth factor (VEGF), a proangiogenic factor associated with tumor angiogenesis and progression, was measured in the plasma of canine osteosarcoma

patients and found to be measurable, whereas normal dog plasma contained no measurable concentrations. The biologic or clinical significance to this finding is not known at this time.[99] Finally, angiostatin, a potent inhibitor of angiogenesis, has been measured in the urine of dogs with primary bone tumors yet was absent once the tumor was excised from the patient.[100] How the production of angiostatin would be involved in local tumor progression and metastasis is still unknown.

As tumor progression is, at least in part, a balance between tumor proliferation and tumor death (Chapter 2), investigations of the proliferative and death (or apoptosis) machinery involved in OS have been investigated. The proliferation marker Ki-67 was found to be overexpressed in canine OS cell lines as compared to chondrosarcoma lines;[101] however, the significance of this is unknown. Several investigations have shown that proliferation and apoptosis can be modified in canine OS cells, both *in vitro* and *in vivo*. Apoptosis, differentiation, and growth inhibition was induced in OS cells by binding retinoic acid receptors (RAR's) and through the addition of bisphosphonates (e.g., zoledronate, pamidronate, alendronate), and these may represent potential therapeutic targets or agents.[102-106] Tumor cell immortalization through alterations in telomere biology (Chapter 14, Section D) have also been investigated in canine OS and in one study the telomerase reverse transcriptase gene was found to be upregulated in a number of tumors, including canine OS.[107]

Several other genetic factors have been implicated in people and dogs with OS. In 33 cases of OS in people, clonal chromosome changes were seen in 17 patients, with supernumerary ring formation being consistent with the parosteal phenotype.[108] Further evaluation of genetic alterations in canine OS are currently ongoing. In order to facilitate the evaluation of gene mutations in canine OSA, microarray techniques have been applied (Chapter 8). More refinement and bioinformatics analysis of these diagnostic tools are currently under way.[109,110] Such data are mounting for human OS and are now being translated to clinical application.[111] Breed agencies and support organizations are presently involved in tissue archiving with a goal of genetic screening for susceptibility prediction.[112]

Pathology and Natural Behavior

Osteosarcoma is a malignant mesenchymal tumor of primitive bone cells. These cells produce an extracellular matrix of osteoid, and the presence of tumor osteoid is the basis for the histological diagnosis, differentiating OS from other sarcomas of bone. The histologic pattern may vary between tumors or even within the same tumor. Small biopsy samples of an OS may lead to misdiagnoses such as chondrosarcoma, fibrosarcoma, hemangiosarcoma, or simply reactive bone.

These histological diagnoses from small biopsies must be interpreted with caution. It is important to obtain a histologic analysis of the entire tumor following definitive excision to confirm the diagnosis. There are many histologic subclassifications of OS based on the type and amount of matrix and characteristics of the cells: osteoblastic, chondroblastic, fibroblastic, poorly differentiated, and telangiectatic osteosarcoma (a vascular subtype). Alkaline phosphatase staining has been shown to aid in differentiating OS pathologically from other connective tissue tumors.[113] In dogs, it has not been well established that there is a difference in the biological behavior of the different histologic subclassifications; however, histologic grade, based on microscopic features, may be predictive for systemic behavior (metastasis).[114] Newer techniques (see previous section) designed to recognize molecular or genetic alterations are being evaluated to determine their potential use in predicting behavior of OS. The degree of aneuploidy, as measured by flow cytometry, of primary and metastatic tumors is potentially indicative of biologic behavior.[115] Osteosarcoma has very aggressive local effects and causes lysis, production of bone, or both processes can occur concurrently (Figure 23-1). The local disease is usually attended by soft tissue swelling. Pathological fracture of the affected bone can occur. OS rarely will cross a joint surface. This confinement to the bone may be secondary to collagenase inhibitors limiting progression through synovium.[116-117] Metastasis is very common and arises early in the course of the disease, although usually subclinically. Although less than 15% of dogs have radiographically detectable pulmonary or osseous metastasis at presentation, approximately 90% will die with metastatic disease, usually to the lungs, within 1 year when amputation is the only treatment.[1,13] Metastasis via the hematogenous route is most common; however, on rare occasions extension to regional lymph nodes may occur.[118] Although the lung is the most commonly reported site for metastasis, tumor spread to bones or other soft tissue sites occurs with some frequency. An increase in the incidence of bone metastasis following systemic chemotherapy has also been documented in humans and is suspected in dogs.[119-122] Possible explanations for this change include a change in the behavior of this cancer independent of treatment; selective killing of metastatic cancer by chemotherapy in certain sites, such as the lung, which allows metastasis in other sites to become clinically relevant; lung resection and chemotherapy have improved survival and bone sites become clinically relevant; more sensitive detection methods, which allow previously undetectable metastases to be seen; or more complete and detailed necropsies compared to those performed previously, which identify asymptomatic metastatic sites. Suspected locoregional (skip) metastasis has also been reported.[123]
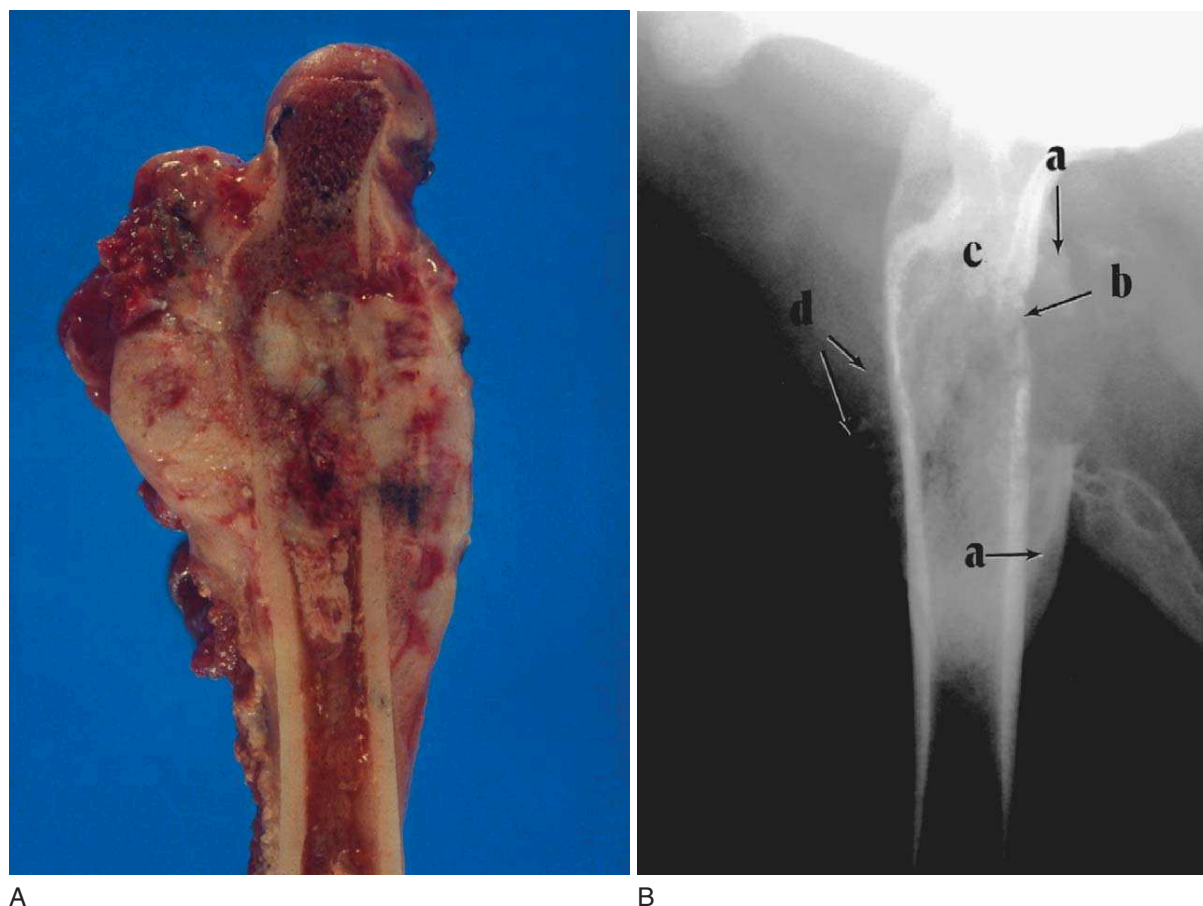
Figure 23-1

A, Gross, longitudinally split specimen of a proximal femoral osteosarcoma lesion in a dog showing cortical destruction, soft tissue, and osteoid neoplastic components. **B,** Lateral radiograph of a proximal femoral osteosarcoma lesion from the case in part **A**. Radiographic features include *(a)* Codman's triangle, *(b)* cortical lysis, *(c)* loss of trabecular pattern in the metaphases, and *(d)* tumor bone extension into the soft tissues in a sunburst pattern.

Some differences in metastatic behavior have been observed based on the anatomic location of the primary OS site. For example, mandibular OS and, to a degree, other calvarium locations may have a less aggressive metastatic behavior, although contradictory evidence exists (see Therapy and Prognosis, presented later in the chapter).[124,125]

There is a report of four cases of histologically confirmed OS that subsequently underwent spontaneous regression without tumor-specific treatment.[126] This phenomenon, while extremely rare, has also been reported in people.

History and Clinical Signs

Dogs with OS of appendicular sites generally present with a lameness and swelling at the primary site. There may be a history of mild trauma just prior to the onset of lameness. This history can often lead to misdiagnosis as another orthopedic or soft tissue injury. The pain is likely due to microfractures or disruption of the periosteum induced by osteolysis of cortical bone with tumor extension from the medullary canal. The lameness worsens and a moderately firm to soft, variably painful swelling arises at the primary site. Dogs may present with acute, severe lameness associated with pathologic fractures, although pathologic fractures account for less than 3% of all fractures seen.[127] Large- and giant-breed dogs that present with lameness or localized swelling at metaphyseal sites should be evaluated with OS as a likely diagnosis.

The signs associated with axial skeletal OS are site dependent. Signs vary from localized swelling with or without lameness to dysphagia (oral sites), exophthalmos and pain on opening the mouth (caudal mandibular or orbital sites), facial deformity and nasal discharge

(sinus and nasal cavity sites), and hyperesthesia with or without neurological signs (spinal sites). Dogs with tumors arising from ribs usually present because of a palpable, variably painful mass. Respiratory signs are not common even where the lesions have large intrathoracic components, and malignant pleural effusion is quite rare.

Dogs rarely have respiratory signs as the first clinical evidence of pulmonary metastasis; rather, their first signs are usually vague. With radiographically detectable pulmonary metastasis, dogs may remain asymptomatic for many months, but most dogs develop decreased appetites and nonspecific signs such as malaise within 1 month. Hypertrophic osteopathy may develop in dogs with pulmonary metastasis (Chapter 5).

Systemic alterations

Alterations in energy expenditure, protein synthesis, urinary nitrogen loss, and carbohydrate flux have been documented in dogs with OSA, similar to results documented in humans with neoplasia. Changes were documented in resting energy expenditure as well as protein and carbohydrate metabolism in dogs with OSA. These changes were evident even in dogs that did not have clinical signs of cachexia.[128] Systemic, metabolic derangements reported for dogs with OS include lower chromium and zinc levels, lower iron and iron binding capacity, and increased ferritin levels as compared to normal dogs.[129] Hypercalcemia is extremely rare. The impact of these changes on patient treatment, response, or outcome is yet unknown.

Diagnostic Techniques and Workup

Radiology

Initial evaluation of the primary site involves interpretation of good quality radiographs taken in lateral and craniocaudal projections. Special views may be necessary for lesions occurring in sites other than in the appendicular skeleton. The overall radiographic abnormality of bone varies from mostly bone lysis to almost entirely osteoblastic or osteogenic changes (see Figure 23-1B).[130] There is an entire spectrum of changes between these two extremes, and the appearance of OS can be quite variable. There are some features, however, that are commonly seen. Cortical lysis is a feature of OS and may be severe enough to leave obvious areas of discontinuity of the cortex leading to pathological fracture. There is often soft tissue extension with an obvious soft tissue swelling, and new bone (tumor or reactive bone) may form in these areas in a palisading pattern perpendicular or radiating from the axis of the cortex (i.e., "sun-burst"). As tumor invades the cortex, the periosteum is elevated and new bone is laid down by the cambium layer providing a triangular appearing deposition of dense new bone on the cortex at the periphery of the lesion.

This periosteal new bone has been called "Codman's triangle," but this is not pathognomonic for osteosarcoma. Osteosarcoma does not directly cross articular cartilage, and primary lesions usually remain monostotic. The tumors may extend into periarticular soft tissues, however, and adjacent bones are at risk because of extension through adjacent soft tissue structures. Other radiographic changes that can attend OS are loss of the fine trabecular pattern in the metaphysis, a vague transition zone at the periphery of the medullary extent of the lesion (rather than a sharp sclerotic margin) or areas of fine punctate lysis. Any one or combinations of these changes may be seen depending on the size, histological subtype, location, and duration of the lesion. The radiographic appearance of OS is similar to osteomyelitis, specifically of fungal etiology.[131-133] In cases where the travel or clinical history might support the possibility of osteomyelitis, a biopsy with submission for histology and culture may be warranted.

Based on signalment, history, physical exam findings, and radiographic findings, a presumptive diagnosis of OS can be made. Differential diagnoses of lytic, proliferative, or mixed pattern aggressive bone lesions identified on radiographs include other primary bone tumors (chondrosarcoma, fibrosarcoma, hemangiosarcoma); metastatic bone cancer; multiple myeloma or lymphoma of bone; systemic mycosis with bony localization; bacterial osteomyelitis; and, albeit rare, bone cysts.

Other primary bone tumors are far less common but may be suspected especially in dogs with unusual signalment or tumor location. Metastatic cancer can spread to bone from almost any malignancy. A careful physical exam is important including a rectal exam with special attention paid to the genitourinary system to help rule out the presence of a primary cancer. Dogs with a history of cancer in the past should have their original biopsy reviewed and should be restaged for the original disease. Common sites for metastatic bone cancer are lumbar and sacral vertebrae, pelvis, and diaphyses of long bones. There are usually other clues for the diagnosis of multiple myeloma such as hyperproteinemia, and both multiple myeloma and lymphoma of bone are usually attended by radiographic lesions that are almost entirely lytic. Two classic radiographic appearances of myeloma bone lesions are described: either "punched-out" areas of lysis or a generalized osteoporetic thinning of cortices.

Tissue biopsy

A diagnosis of primary malignant bone tumor may be suggested by signalment, history, physical examination, and radiographic findings. Cytology has not been thought to be definitive for diagnosis; however, it may support the tentative diagnosis, and, combined with clinical features and radiographic appearance, enough confidence in the diagnosis to move forward with

discussion of treatment options may exist. Consistent cytologic criteria of OS has recently been described, and with repeated evaluations, dependent on experience, cytopathologists may be more definitive in making a diagnosis from cytology alone.[134,135] Alkaline phosphatase staining of cytological samples may help to differentiate OS from other vimentin-positive tumors.[113] However, in most cases, a definitive diagnosis lies in procurement and interpretation of tissue for histopathology. With new treatments such as limb sparing (see subsequent sections), knowledge of the specific tumor type may avoid overextensive or inappropriate treatment of bone tumors thought to be osteosarcoma (e.g., chondrosarcoma, myeloma, or lymphoma). It is crucial to the success of a limb-sparing surgery that the biopsy procedure is planned and performed carefully with close attention to asepsis, hemostasis, and wound closure.[136] The skin incision for the biopsy must be small and placed such that it can be completely excised with the tumor at limb sparing without compromising the procedure. Transverse or large incisions must be avoided. It has been recommended that the surgeon who is to perform the definitive surgical procedure (especially if this is limb sparing) should be the person to perform the preoperative bone biopsy.[137]

Bone biopsy may be performed as an open incisional, closed needle, or trephine biopsy (Figures 23-2 and 23-3). The advantage of the open techniques is that a large sample of tissue is procured, which presumably improves the likelihood of establishing an accurate histological diagnosis. Unfortunately, this advantage may be outweighed by the disadvantages of an involved operative procedure, and risk of postsurgical complications such as hematoma formation, wound breakdown, infection, local seeding of tumor, and pathological fracture.[138,139] Although closed biopsy with a Michelle trephine yields a diagnostic accuracy rate of 93.8%, there is increased risk of creating pathological fracture than with a smaller gauge needle.[140] This underscores some of the advantages of a closed biopsy using a Jamshidi bone marrow biopsy needle or similar type of needle.* Jamshidi needle biopsy has an accuracy rate of 91.9% for detecting tumor versus other disorders and an 82.3% accuracy rate for diagnosis of specific tumor subtype.[141] Accuracy of diagnoses from needle core samples can be dependent on the pathologist's experience and comfort level with examination of small samples.

The biopsy site is selected carefully. Radiographs (two views) are reviewed and the center of the lesion chosen for biopsy. Biopsy at the lesion periphery will often result in sampling the reactive bone surrounding the tumor growth without a resulting diagnosis.[141]

*Jamshidi bone marrow needle, American Pharmaseal Co., Valencia, CA. Bone marrow biopsy needle, Sherwood Medical Co., St. Louis, MO.

The skin incision is made so the biopsy tract and any potentially seeded tumor cells can be completely removed at the time of definitive surgery. Care is used to avoid major nerves, vessels, and joint spaces. A 4-inch, 8- or 11-gauge needle is used. With the dog anesthetized, prepared, and draped for surgery, a small stab incision (2 to 3 mm) is made in the skin with a #11 scalpel blade. The bone needle cannula, with the stylet locked in place, is pushed through the soft tissue to the bone cortex. The stylet is removed, and the cannula is advanced through the bone cortex into the medullary cavity using a gentle twisting motion and firm pressure. The opposite cortex is not penetrated. The needle is removed and the specimen is gently pushed out of the base of the cannula by inserting the probe into the cannula tip. One or two more samples can be obtained by redirecting the needle through the same skin incision so that samples of the transition zone may also be obtained. Ideal specimens should be 1 or 2 cm in length and not fragmented. Biopsy is repeated until solid tissue cores are obtained. Material for culture and cytology may be taken from the samples prior to fixation in 10% neutral buffered formalin. Diagnostic accuracy is improved when samples are evaluated by a pathologist thoroughly familiar with bone cancer. Fluoroscopy or advanced imaging (CT) can assist in obtaining needle-core biopsy samples of suspected bone lesions, especially for axial sites.[142]

After tumor removal (amputation or limb sparing), histology should be performed on a larger specimen to confirm the preoperative diagnosis. If the clinical and radiographic features are typical for OS, especially when there is little possibility of fungal or bacterial infection, confirmation of histologic diagnosis following surgical treatment of local disease (amputation or limb sparing) can be considered. Few diseases causing advanced destruction of the bone can be effectively treated without removal of the local disease. If the owners are willing to treat aggressively, surgical removal of local disease with biopsy submission following surgery may be acceptable.

Staging and patient assessment

Systemic Staging. Examination for evidence of apparent spread of the disease is important. Regional lymph nodes, although rarely involved, should be palpated and fine needle cytology performed on any enlarged node.[118] Sites of bone metastasis may be detected by a careful orthopedic examination with palpation of long bones and the accessible axial skeleton. Organomegaly may be detected by abdominal palpation. Usually pulmonary metastases are undetectable by clinical exam, but careful thoracic auscultation is important to detect intercurrent cardiopulmonary disorders. High detail thoracic radiographs should be taken during inspiration with the patient awake. Although some controversy exists,[143] it is still considered
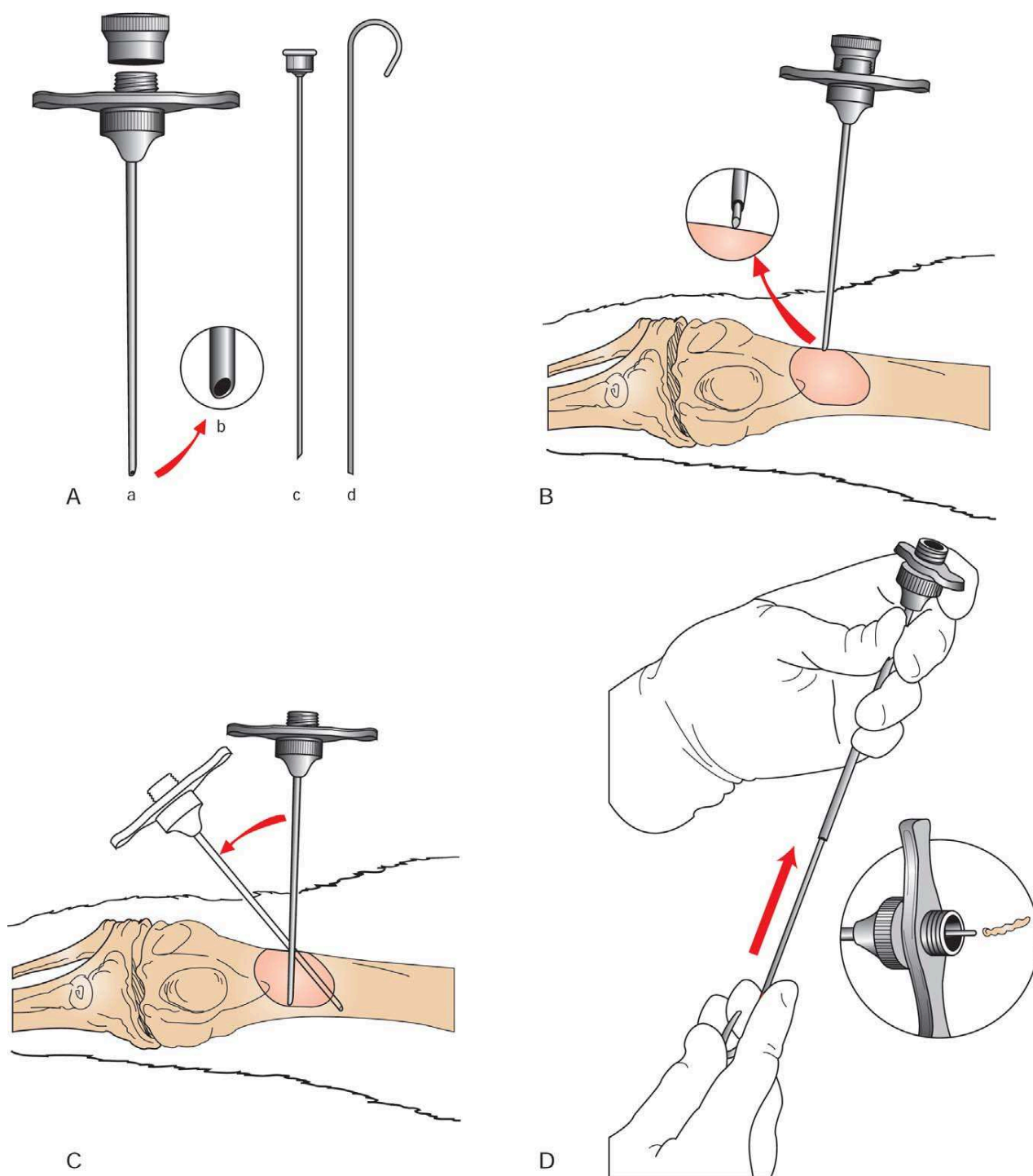
Figure 23-2

A, The Jamshidi bone biopsy needle: cannula and screw-on cap (**D**), tapered point (**B**), pointed stylet to advance cannula through soft tissues (**C**), and probe to expel specimen from cannula (**D**). **B**, With the stylet locked in place, the cannula is advanced through the soft tissue until bone is reached. The inset is a closeup view showing stylet against bone cortex. **C**, The stylet is removed and the bone cortex penetrated with the cannula. The cannula is withdrawn and the procedure repeated with redirection of the instrument to obtain multiple core samples. **D**, The probe is then inserted retrograde into the tip of the cannula to expel the specimen through the base (inset). *(Reprinted with permission from B. E. Powers, S. M. LaRue, S. J. Withrow, R. C. Straw, et al., "Jamshidi needle biopsy for diagnosis of bone lesions in small animals," J Am Vet Med Assoc 193:206–207, 1988.)*

Figure 23-4

Scintigraphic view of a distal radial osteosarcoma lesion in a dog following $^{Tecnetium-99M}$-hydroxymethylene diphosphonate injection. (A) Uptake within the tumor. (B) Mild uptake within the elbow joint secondary to degenerative joint disease.

important by most oncologists to include three views: a ventro-dorsal or a dorso-ventral view and both right and left lateral views. Osteosarcoma pulmonary metastases are generally soft tissue dense and cannot be detected radiographically until the nodules are 6 to 8 mm in diameter. It is uncommon to detect pulmonary metastatic disease at the time of diagnosis (less than 10% of dogs). Advanced imaging (e.g., CT, MRI, PET/CT) may play a role in patient staging and is used to evaluate for pulmonary metastases and for evaluation of tumor vascularity, soft tissue and medullary involvement, and response to treatment in people.[144-145] Currently, published treatment recommendations and prognoses are based on the results of plain radiographs. As advanced imaging becomes more commonplace for staging dogs with OS, comparisons to previous protocols will be subject to stage-migration and lead-time bias as earlier diagnosis will result.

Bone survey radiography has been useful in detecting dogs with second skeletal sites of osteosarcoma.[34] Bone surveys include lateral radiographs of all bones in the body and a ventro-dorsal projection of the pelvis using standard radiographic technique appropriate for the region radiographed. One hundred and seventy-one dogs with primary bone tumors underwent radiographic bone surveys and thoracic radiography in one study, and at presentation, there was a higher yield in finding other sites of OS with radiographic bone survey (6.4%, 11 of 171 dogs) than with thoracic radiographs (4%, 7 of 171 dogs).[146] There are conflicting reports on the usefulness of nuclear scintigraphy (bone scan; Figure 23-4) for clinical staging of dogs with osteosarcomas.[147-151] Bone scintigraphy was used in one study to identify suspected second bone sites of OS in 14 of 25 dogs with appendicular primaries.[148] Seven of these lesions were biopsied and confirmed to be osteosarcoma. Another study of 70 dogs with appendicular primary bone tumors resulted in only one scintigraphically detectable occult bone lesion.[149] In a third report, of 23 dogs with suspected skeletal neoplasia that were evaluated with scintigraphy and radiography, 4 dogs had second skeletal sites suspected to be neoplastic.[150] The suspicious site in one of these dogs was found on histological evaluation to be normal bone. Another study found secondary sites considered highly suspect of bony metastasis in 7.8% of (399) cases; however, most suspected lesions were not subjected to histologic conformation.[151] Nuclear bone scan can be a useful tool for the detection and localization of bone metastasis in dogs presenting for vague lameness or signs such as back pain and while very sensitive, they are not specific for identifying sites of skeletal tumor. Any region of osteoblastic activity will be identified by this technique including osteoarthritis

and infection. Follow-up with high detail radiographs of sites found suspicious on scintigraphy will generally help rule out non-neoplastic disease; however, definitive biopsy may be necessary.

Surgical Staging. A surgical staging system for sarcomas of the skeleton has been devised for people.[152] This system is based on the histological grade (G), the anatomic setting of the primary tumor (T) and regional or distant metastasis (M). There are three stages: stage I, the low-grade (G_1) lesions without metastasis; stage II, the high-grade (G_2) lesions without metastasis; and stage III, the lesion with regional or distant metastasis regardless of histological grade. The stages are subdivided by the anatomic setting, *A* being intracompartmental (T_1) and *B* extracompartmental (T_2). According to this system, most dogs with OS present with stage IIB disease. Scintigraphy can be used to evaluate the degree of bone involvement from a primary bone tumor.[153] In one study, scintigraphy overestimated the length of OS disease in limb spare patients by 30%, allowing for adequate margin prediction, preoperatively.[154] Computed tomography may be useful to plan surgery, especially for tumors located in the axial skeleton; however, one study reported that plain radiographs were as accurate as advanced imaging (CT, MRI) in predicting true length of tumor involvement.[155] In contrast, MRI was more accurate than plain radiographs or CT in predicting length of tumor involvement for appendicular canine OS in another study.[156] This could prove invaluable for limb-sparing patients, and further evaluation is warranted.

Patient Assessment. The patient's overall health status requires careful assessment. Advancing years do not preclude treatment; however, prolonged anesthesia and chemotherapy may not be tolerated in dogs with organ compromise. Particular attention to the cardiovascular system is important. Coexisting cardiomyopathy or any degree of heart failure may lead to serious complications, particularly during fluid diuresis, anesthesia, or administration of certain chemotherapy agents. An electrocardiogram and echocardiogram should be performed on dogs where the history or physical findings implicate a cardiac disorder. Renal function must be evaluated prior to administration of cisplatin. A minimum database should include a complete blood count, platelet count, serum biochemical analysis, and urinalysis.

Known or Suggested Prognostic Factors

Anatomic location and signalment
The biologic behavior for nonappendicular sites of OS appears to be similar (aggressive) with the exception of the mandible and possibly the rest of the calvarium.[124,125,157-159] Dogs with OS of the mandible treated with mandibulectomy alone had a 1-year

survival rate of 71% in one study. [124,] In contrast, maxillary OS has demonstrated a median survival of 5 months following maxillectomy.[160-162] A study evaluating response to treatment for orbital OS reported long-term survival following complete surgical excision.[163] Similar behavior is seen for OS of flat bones in people.[164,165] Median survival for rib OS lesions is reported to be 3 months for dogs treated with rib resection alone and 8 months for dogs treated with resection and adjuvant chemotherapy.[166-169]

Osteosarcoma of the canine scapula has been reported to have a poor prognosis when treated with surgery and chemotherapy.[170,171] Survival of dogs with OS distal to the antebrachiocarpal or tarsocrural joints was somewhat longer (median of 466 days) than survival of dogs with OS of more common appendicular sites; however, OS in these sites is aggressive with a high potential for metastasis.[31]

Extraskeletal (soft tissue) OS sites also appear to have aggressive systemic behavior. In one report, extraskeletal OS treated with surgery alone had a median survival of only 1 month, and a median survival of 5 months was obtained for cases treated with surgery and adjuvant chemotherapy.[38] In a larger study, soft tissue OS were separated from mammary gland OS; median survival of nonmammary gland soft tissue lesions was 1 month and mammary gland lesions 3 months following primarily surgical resection alone.[39]

Vertebral OS is uncommon; however, reported cases indicate aggressive local and systemic behavior.[26,172] In 15 dogs treated with a combination of surgery, radiation, and chemotherapy, the median survival was 4 months.[173] The biologic behavior of OS in other nonappendicular sites has not been thoroughly evaluated. Although there are differences in disease distribution and prevalence, documentation of improved survival for small dogs with OS is lacking.[174]

In a multi-institutional study of 162 dogs with appendicular OS treated with amputation alone, dogs younger than 5 years of age had shorter survival than older dogs.[13] Additional studies have related large tumor size[11,175] and humerus location[176] to poor outcome. Large tumor size has been reported to be a negative prognostic factor for people with osteosarcoma.[177] For OS originating from flat bones, small dog size and completeness of excision were positive prognostic indicators.[170] A negative prognosis can also be predicted by a higher tumor grade, based on the results of one study.[114] Dogs presented with stage III disease (measurable metastases) have a very poor prognosis, and dogs with lymph node metastasis had short survivals with a median of only 59 days, compared to 318 days for dogs without nodal spread.[118]

Serum Alkaline Phosphatase. Elevated alkaline phosphatase has been clearly associated with a poorer prognosis for dogs with appendicular OS in several studies.[114,118,178-180] A preoperative elevation of either

total (serum) or the bone isoenzyme of AP (greater than 110U/L or 23U/L, respectively) is associated with a shorter disease free interval and survival (Figure 23-5). Likewise, dogs that have elevated preoperative values that do not return to normal within 40 days following surgical removal of the primary lesion also fail earlier from metastasis. One study substantiated the predictive nature of elevated preoperative AP levels; however, no association was found for elevated postoperative serum levels.[179]

Molecular/Genetic Indices of Prognosis. Measurement of p53 mutations have been associated with the presence of the multiple drug resistance gene (MDR1)[181] and have been shown to predict cisplatin resistance *in vitro*.[182] The presence of drug resistance through the measurement of MDR1 or indirectly by the measurement of p-glycoprotein (PGP) has been loosely associated with worse outcome in human patients with osteosarcoma and high-grade chondrosarcoma.[183-185] Evaluation of the role of PGP and outcome in canine OS is currently under investigation. Other work has also identified heat shock protein 72 to be predictive of good response to neoadjuvant chemotherapy for OS in people.[186] Increases in molecular measures of tumor cell proliferation rate, including the potential doubling time (T_{pot}) or MIB-1 expression, may be indicators of more aggressive tumor cell kinetics and have been associated with a worse prognosis.[101,187] One report evaluated the presence of overexpression of cyclooxygenase-2 (COX-2) in 44 canine appendicular OS and found 34 cases showing strong expression had a significantly poorer outcome.[95] Finally, the presence of ezrin, a membrane-cytoskeleton linker, has been shown to be predictive of metastatic behavior and survival in dogs with OS.[94]

Therapy Directed at the Primary Tumor

Surgery

Table 23-2 provides an overview of surgical options for primary bone tumors based on anatomic site.

Amputation. Amputation of the affected limb is the standard local treatment for canine appendicular osteosarcoma. Even large- and giant-breed dogs will usually function well after limb amputation, and most owners are pleased with their pets' mobility and quality of life after surgery.[188,189] Even moderate preexisting degenerative joint disease at the level found in most older, large-breed dogs is rarely a contraindication for amputation. Most dogs will readily compensate, and although the osteoarthritis may progress more rapidly in the three-legged dog, this rarely results in a clinical problem. Severe preexisting orthopedic or neurological conditions may cause poor results in some cases, and careful preoperative examination is important. A complete forequarter amputation for forelimb lesions is generally recommended, as is a coxofemoral disarticulation
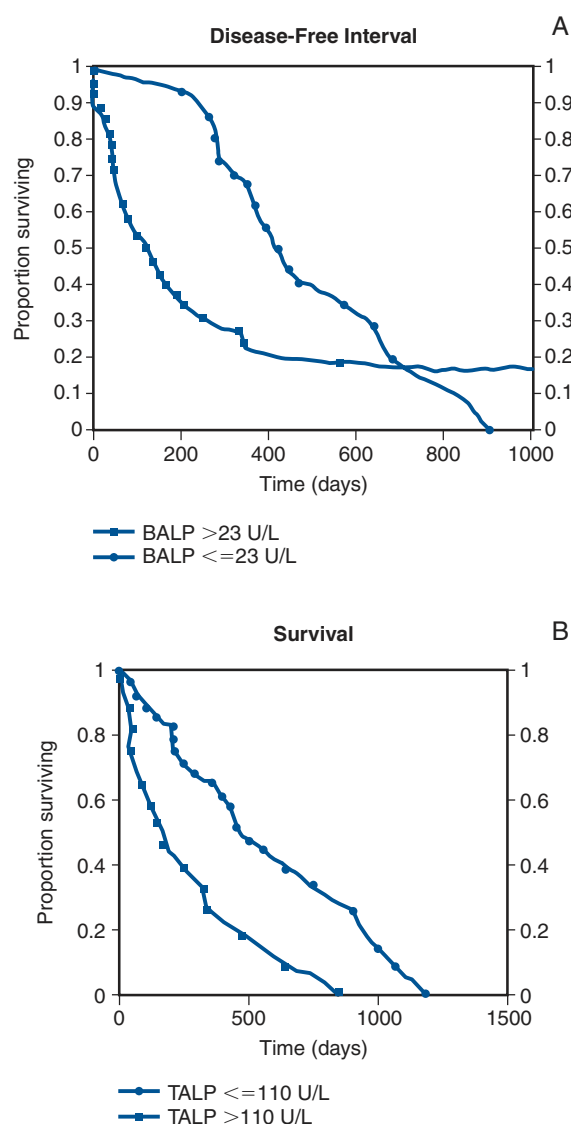
Figure 23-5

A, Disease-free interval outcome of dogs treated for osteosarcoma comparing bone alkaline phosphatase levels above and below 23 U/L preoperatively. **B,** Survival outcome of dogs treated for osteosarcoma comparing serum alkaline phosphatase levels above and below 110 U/L preoperatively. *(Reprinted with permission from N. Erhart, W. S. Dernell, W. E. Hoffmann, R. M. Weigel, B. E. Powers, and S. J. Withrow, "Prognostic importance of alkaline phosphatase activity in serum from dogs with appendicular osteosarcoma: 75 cases (1990–1996)," J Am Vet Med Assoc 213:1003, 1998.)*

amputation for hind leg lesions. This level of amputation assures complete local disease removal and also results in a more cosmetic and functional outcome. For proximal femoral lesions, a complete amputation and en bloc acetabulectomy is recommended to obtain

TABLE 23-2   Treatment Options for Osteosarcoma by Site

Site	Treatment Options	Comments
Humerus, femur, tibia	Amputation Limb-spare in limited cases	Generally high complication rate for limb spare[175] Diaphyseal locations amenable to intercalary allografts[197] Total-hip spare possible for proximal femoral lesions[204] Intraoperative extracorporal radiation technique may apply[213]

Radius	Amputation
	Limb spare
	    allograft[190-195]endosteal
	    prosthesis[204] intercalary
	    bone graft[197] ulnar
	    transposition[211,212] bone
	    transport osteogenesis[207-210]
	    pasteurized autograft[205,206]
	    intraoperative extracorporal
	    radiation therapy[213]
		Can combine radius/ulna resection (graft radius only)

Ulna	Amputation Ulnectomy[229]	Often does not require allograft reconstruction
Scapula	Amputation Scapulectomy[171,223]	Proximal lesions best; complete scapulectomy described

Pelvis	Pelvectomy with or without
	    amputation[222]
		Lateral portion of sacrum can be excised; may include
		    body wall

Metacarpus/metatarsus	Amputation[30] Local resection[31]	Limb-spare function dependent on bone(s) involved
Mandible	Mandibulectomy[224]	Often requires total hemimandibulectomy Bilaterally limited to fourth premolar
Maxilla/orbit	Maxillectomy[225,226] Orbitectomy[228]	Limited by midline palate or cranial vault invasion Combined approach may assist exposure
Calvarium	Resection +/− Radiation	Resection dependent on venous sinus involvement

Vertebrae	Decompression (palliative)
	    +/− Radiation/Chemo[173]
		Vertebrectomy techniques not well developed; limited local
		    disease control

Rib	Rib resection[166-168]	Requires removal of additional cranial and caudal rib

proximal soft tissue margins (Figure 23-6). Surgery alone must be considered palliative for osteosarcoma as microscopic metastatic disease is present in the vast majority of cases at diagnosis and amputation does not address these.

Limb-Sparing Surgery. Although most dogs function well with amputation, there are some dogs where limb sparing would be preferred over amputation, such as dogs with severe preexisting orthopedic or neurological disease or dogs with owners who absolutely will not permit amputation. Until recently, only a few reports of limb sparing in dogs, with limited follow-up, have appeared in the literature.[190-194] To date, more than 500 limb-sparing procedures have been performed at Colorado State University' Animal Cancer Center (CSU-ACC). Limb function has generally been good to excellent in most dogs,

and survival has not been adversely affected by removing the primary tumor with marginal resection.[195]

Suitable candidates for limb sparing include dogs with osteosarcoma clinically and radiographically confined to the leg, where the primary tumor affects <50% of the bone (as determined radiographically) and dogs that are in otherwise good general health. Other criteria for consideration include absence of pathologic fracture, less than 360° involvement of soft tissues, and a firm/definable soft tissue mass *versus* an edematous lesion. Early on in the development of limb-sparing procedures, many dogs treated at CSU-ACC received some form of preoperative treatment, (i.e., primary or neoadjuvant intra-arterial [IA] cisplatin, intravenous [IV] cisplatin, radiotherapy to the tumor bone, or a combination of radiotherapy with IV or IA cisplatin).

Figure 23-6
A, Ventro-dorsal radiographic view of an osteosarcoma of the ilium of a dog. **B,** Ventro-dorsal radiographic view of the dog in **A** 3 years after hemipelvectomy and amputation followed by cisplatin chemotherapy.

Results from 21 dogs treated with radiation therapy alone given in large doses per fraction prior to limb sparing were unsatisfactory for preservation of life or limb.[193] Many of the dogs treated with two preoperative IA cisplatin doses 21 days apart, with the last treatment 21 days prior to surgery, showed marked decrease in the degree of vascularization of the tumor. This represented a high degree of induced tumor necrosis in the resected specimen, especially when combined with radiation therapy, and facilitated limb sparing.[194,196] Currently at CSU-ACC, case selection predetermines the use of local chemotherapy or preoperative downstaging for limb-sparing cases, and most dogs receive systemic cisplatin, carboplatin, doxorubicin, or combination therapy after surgery (see Local Adjuvant Therapies Used Concurrent to Limb Salvage, presented later in the chapter).

The most suitable cases for limb sparing are dogs with tumors in the distal radius or ulna as function following limb sparing and carpal arthrodesis is good. Arthrodesis of the scapulohumeral, coxofemoral, stifle,

or tarsal joints following limb sparing generally results in only fair to poor function.[175] Resulting poor function, combined with a high complication rate, has generally led surgeons away from recommending limb sparing near these joints. Limb sparing is a complicated process and requires a coordinated team effort between surgical and medical oncologists, radiologists, pathologists, and technical staff. Several methods of limb sparing have been described, each with unique advantages and limitations. The choice of limb-sparing method depends on several factors including owner choice, patient personality, and individual risk factors. At the CSU-ACC, owners are given a choice of limb-sparing procedures and informed about the risks and benefits of each method compared with amputation. A brief description of the surgical options for a distal radial location (most common) follows. In all cases, cephalosporin antibiotics are administered intravenously immediately preoperatively, intraoperatively, and for 24 hours postoperatively. Meticulous aseptic technique is essential.

Allograft Limb Sparing. For a distal radial site, the dog is placed in lateral or dorsal recumbency, with the affected limb uppermost. A skin incision is made on the dorsolateral aspect of the antebrachium from a point just distal to the elbow, to just proximal to the metacarpophalangeal joint. Any biopsy tracts are excised *en bloc*. Soft tissue is dissected to the level of the tumor pseudocapsule. Care is taken not to enter the tumor. The bone is cut with an oscillating bone saw 3 to 5 cm proximal to the proximal radiographic (or scintigraphic) margin of the tumor. Extensor muscles attached to the tumor pseudo capsule are transected at a level to maintain 2 to 3 cm soft tissue margins. The joint capsule is incised, keeping close to the proximal row of carpal bones. For tumors of the mid diaphysis, tumor resection follows similar guidelines with the exception that an attempt to spare extensor and flexor muscle groups is undertaken as the joint (above and below) may be spared.[197] The ulna is sectioned sagittally with an osteotome and the medial ulnar cortex adjacent to the tumor is removed *en bloc* with the radius. For tumors that have extension to the ulna (rare), the ulna is also cut with a bone saw and the distal one-third or more is removed with the tumor. Care is taken to preserve as much vasculature as possible, especially on the palmar surface. Large vessels associated with the tumor are ligated and divided. Surgical hemostatic staples* are very useful. The specimen is radiographed, then submitted for histologic evaluation, including assessment of completeness of surgical margins and percentage of tumor necrosis. In addition, a sample of bone marrow proximal to the resection level (radius) is obtained for histologic evaluation of marrow involvement. Intraoperative frozen section histology is used in people to assess the adequacy of surgical resection of primary bone tumors during limb sparing.[198] Although this technique is being used in veterinary medicine, it is still considered somewhat unreliable for bone specimens.

A fresh-frozen cortical allograft is thawed in 1 liter of an antibiotic in saline solution, the articular cartilage is removed, the graft is cut to fit,† and the medullary cavity reamed to remove fat and cellular debris.[199,200] The articular cartilage of the proximal carpal bones is removed, and the allograft is stabilized in compression using Association for the Study of Internal Fixation (ASIF/AO) principles. A dynamic compression plate with a minimum of three screws proximal and four screws distal to the graft is used; 3.5 mm broad plates of up to 22 hole size or a custom-designed limb salvage plate are appropriate in most cases, but for very large dogs, 4.5 mm narrow or broad plates are selected. The plate is fastened in the patient to the allograft with two

or three screws, removed from the surgery site, and the medullary canal of the allograft is filled with polymethyl methacrylate* bone cement containing amikacin (1 g amikacin to 40 g of polymer powder). This provides support for the screws during revascularization of the graft and acts as a reservoir for antibiotics. The healing of the allograft is not significantly impeded by the presence of the cement and has been shown to significantly decrease the incidence of orthopedic failure, including allograft fracture and screw pullout.[201,202] The plate extends proximally in the host radius and distally to a level just proximal to the metacarpophalangeal joint (Figure 23-7). For intercalary limb spares, the plate extends proximally and distally to meet or exceed ASIF standards with the intent to spare joint motion.

The wound is thoroughly lavaged with saline and it is at this point that local (polymer) chemotherapy may be implanted. A closed suction drain is inserted adjacent to the allograft, and the wound is closed. The leg is supported in a padded bandage. The drain is removed the day after surgery in most cases. It is most important to prevent self-mutilation (licking) after surgery, and Elizabethan collars should be used as necessary. No external coaptation is used and most dogs use the limb fairly well by 10 days after surgery. Postoperative foot swelling can be considerable but usually resolves by 2 weeks. Although decreased exercise is recommended for the first 4 to 6 weeks to allow soft tissues to heal, no exercise restriction need apply after this time. In fact, it is important that limb use is encouraged, even in early postoperative times, so that flexure contracture of the digits does not occur. Early weight bearing will often decrease the occurrence and incidence of postoperative swelling.

The advantages to allograft limb sparing include the absence of external fixation and little owner involvement is required in the postoperative period aside from bandage changes in the first 2 weeks. The disadvantages are the high infection rate and the need for permanent internal hardware. Canine limb spare patients have an infection rate of approximately 40% and 50%. Once an infection occurs, it may be controlled with long-term antibiotic therapy but is rarely, if ever, resolved.[203] Infection may result in soft tissue defects from draining tracts, exposure of the plate or allograft, and hardware loosening. Revision surgeries, either for hardware complications or soft tissue reconstruction, are not uncommon. Additionally, amputation for catastrophic implant failure, local recurrence, or unmanageable infection is sometimes required.

Metal endoprosthesis limb sparing. This technique utilizes a commercially available metal endoprosthesis with a modified bone plate (Figure 23-8). The surgery is

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A   B   C

Figure 23-7

Limb Sparing. **A,** Preoperative lateral radiograph of a distal radial osteosarcoma lesion in a dog. **B,** Craniocaudal specimen radiograph following tumor resection of the case in part **A**. **C,** Lateral postoperative radiograph following allograft replacement and plate fixation of the case in parts **A** and **B**.

nearly identical to the procedure described earlier; however, instead of reconstruction with an allograft, the endoprosthesis is used to span the radial defect. A prospective comparison of complications between allograft limb sparing and metal endoprosthesis limb sparing has yet to be completed. An advantage to the metal endoprosthesis technique is that large cortical allografts are not required, making limb sparing available to more patients. A technique for limb salvage in patients with tumors of the proximal femur, involving a proximal

femur allograft through which a custom long-stem femoral prosthesis is placed for a total hip replacement, has been used in two patients to date with technical success.[204] This technique is used in people for tumor and nontumor disease of the proximal femur diaphysis or femoral head and neck.

Pasteurized tumoral autograft. Two reports exist of a limb sparing technique that involves removal of the segment of bone with the tumor and pasteurizing the
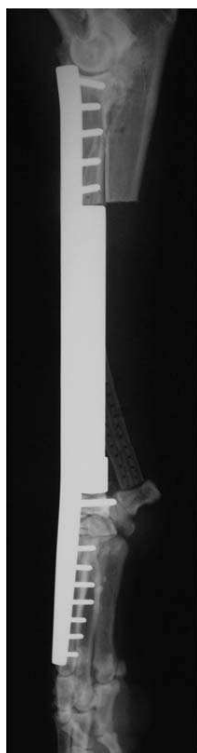
Figure 23-8

Lateral radiographic projection of a limb-sparing technique using a commercially available metal endoprosthesis with a modified bone plate in a dog with distal radial and ulnar osteosarcoma. A limb-sparing plate spans host radius and metacarpus, connecting to the implant, which abuts the host radius proximally and the radial carpal bone distally. A negative suction drain has also been placed at the surgical site to decrease postoperative fluid accumulation.

Figure 23-9

Lateral radiographic projection of a limb-sparing technique known as longitudinal bone transport osteogenesis (BTO). In this case, a distal radial osteosarcoma was removed and BTO was accomplished using circular fixators and the principles of distraction osteogenesis to create bone in the defect remaining following tumor resection. Briefly, a longitudinal section of normal bone (**A** termed the "transport segment") from the radius osteotomized and attached to the transport ring and the osteotomized bone segment is slowly transported into the defect at a rate of 1 mm per day. Distraction osteogenesis occurs in the trailing distraction pathway (**B**).

bone segment at 65 degrees Celsius for 40 minutes, followed by reimplantation.[205,206] Limb function was good in 12 of 13 dogs with a 15% local recurrence, 31% infection, and 23% implant, failure rate. The advantages of this method are that there is no need for an allograft and anatomic apposition is excellent. The disadvantages are similar to the allograft technique in terms of complications; however, overall survival and disease-free progression was similar to other studies.

Longitudinal bone transport osteogenesis (BTO). This technique for limb sparing has been reported in veterinary patients (Figure 23-9).[207,208] This method utilizes Ilizarov (circular) fixators and the principles of distraction osteogenesis to create bone in the defect following tumor resection. Prior to surgery, a five- to six-ring circular fixator is constructed to allow one central ring (termed a *transport ring*) to move independently of the rest of the fixator. Following the same procedure for removal of the tumor and preparation of

the radiocarpal bone described earlier, the circular fixator is placed on the limb and attached to the remaining radius using tensioned 1.6 mm diameter wires. A longitudinal section of normal bone (termed the *transport segment*) from the radius immediately proximal to the defect is osteotomized and attached to the transport ring with wires. Following a 3 to 7 day delay period, the osteotomized bone segment is slowly transported into the defect at a rate of 1mm per day. Distraction osteogenesis occurs in the trailing distraction pathway. New bone continues to form longitudinally within the defect proximal to the transport segment for as long as the steady, slow distraction continues. When the transport segment reaches the radiocarpal bone (*docking*), the transport segment is compressed to the radiocarpal bone and heals to create an arthrodesis. The circular fixator remains on the limb while the newly formed bone remodels and the arthrodesis occurs. This technique is compatible with cisplatin, carboplatin, and combination chemotherapy.[207,209]

The advantages to BTO limb sparing are the lack of internal hardware, the low risk of infection due to the autologous, vascularized nature of the replacement bone, and the ability of the new bone tissue to remodel over time. Patients are typically weight bearing within the first 48 hours and once the incision is healed do not require exercise restriction. The disadvantages of the BTO procedure is the extensive client involvement needed to perform the daily distractions on the fixator and the extended amount of time the fixator remains on the limb. Double level longitudinal transport and translational transport of the ulna can significantly diminish the time required for distraction and has been used successfully in a case of limb salvage for a distal tibial OS.[210]

Ulna transposition limb sparing. The vascularized ulna transposition technique uses the ipsilateral distal ulna as an autograft to reconstruct the distal radial defect by rotating the graft into position while preserving the caudal interosseous artery and vein.[211,212] Following excision of the tumor as described previously, two transverse osteotomies of the ulna are made. The distal osteotomy is performed at the level of the isthmus proximal to the facet that articulates with the radius. The proximal ulna osteotomy is performed 1 to 2 mm distal to the level of the radial osteotomy. Direct visualization of the caudal interosseous artery and vein allows these structures to be preserved during dissection of the autograft. The ulna graft is "rolled over" into the radial defect and fixed using a bone plate that extends from the proximal radius to the distal one third of metacarpal IV (i.e., carpal arthrodesis).

Advantages to the ulna transposition technique are that there is no distant donor site morbidity, the replacement bone is autologous, and the graft is vascularized making it less likely to get infected, and possibly speeding healing. The disadvantages to this technique are that the ulna transposition technique may be more prone to biomechanical complications in the postoperative period due to its smaller size relative to the radius and the need for permanent internal hardware.[212]

Intraoperative extracorporal radiation. This technique for limb sparing has been utilized in a small number of canine osteosarcoma patients.[213,214] This involves osteotomy above or below the affected site (depending on the anatomic location of the tumor) and removal of normal soft tissues from the tumor bone. The neurovascular bundle, muscle, and skin are held away from the affected bone, and the tumor is pivoted from the site on the intact joint tissue. A single dose of 70-Gy radiation is then directed to the tumor, taking care to spare the distracted neurovascular bundle. The radiated bone is then anatomically replaced and fixed back into position using either an interlocking nail system, dynamic compression plating, or a combination. The advantage to this technique is in sparing of immediate joint function, the major limiting factor to the success of limb sparing in nondistal radius or ulna sites. To date, three proximal humeral sites, one distal humeral site, one distal femur, one proximal femur, five distal radius, and three distal tibia sites have been treated with this technique. All patients have had good function in the immediate postoperative period; however, seven patients have had to have implant revisions within 5 to 9 months of initial surgery, including four amputations. Additionally, local tumor recurrence has occurred in four patients and infection in four patients. Modifications of this technique include removing rare tumors of the diaphysis and performing extracorporeal radiation prior to reimplantation. *In situ* radiation of distal femur and any tibial tumors can be performed without osteotomy. Follow-up on this subset is relatively short and further evaluation as well as technique modification is indicated before this technique can be recommended.[213]

Local Adjuvant Therapies Used Concurrent to Limb Salvage

Since 1993, most cases of limb sparing at CSU have received a local polymer chemotherapy implantation at the time of surgery.[215,216] The system is a biodegradable polymer called open cell polylactic acid (OPLA)* containing cisplatin (OPLA-Pt). In a randomized trial evaluating the effect of local implantation OPLA-Pt in 80 cases, recurrence was diagnosed in 32.5% of dogs in the OPLA-Pt group and 60% of dogs in the control group, which approached statistical significance ($p = 0.071$).[216]

Isolation of limb circulation and perfusion with chemotherapy has been used in people with sarcomas and melanomas as a sole treatment or to downstage local disease and allow limb sparing.[217] Isolated limb perfusion (ILP) allows delivery of high concentrations of chemotherapy as well as delivery of compounds that are poorly tolerated systemically. Varying degrees of local toxicity are reported dependent on the drugs used. Successful use of ILP in canine OS has been reported.[218] One study determined that appendicular bone tumors have significantly higher interstitial fluid pressure and lower blood flow than do adjacent, unaffected soft tissues.[219] ILP may be a method to facilitate therapeutic drug concentrations in primary tumors for preoperative downstaging prior to limb salvage. Currently, investigations delivering the radioisotope samarium at high doses using ILP are being conducted in dogs to assess whether a clinically meaningful percentage of necrosis can be achieved prior to primary tumor removal.

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*Open cell polylactic acid, THM Biomedical, Duluth, Minnesota.

Summary of outcome following limb salvage for dogs with OS

There is no significant difference in survival rates for dogs treated with amputation and cisplatin compared to dogs treated with limb sparing and cisplatin. Overall, limb function has been satisfactory, with approximately 80% of dogs experiencing good to excellent limb function.[195] Limb sparing requires a dedicated owner and clinical team. Limb-sparing surgery is usually combined with some form of adjuvant therapy and complications can arise in any or all phases of treatment (chemotherapy, radiation, or surgery). High-dose external beam radiation therapy may complicate wound and bone healing and potentiate infection.[193] Moderate-dose external beam radiation in combination with chemotherapy may, however, be useful for control of local disease, as indicated by percentage of tumor necrosis data.[194,220] The major complications related to surgery are recurrent local disease and allograft infection. In a review of 220 limb-sparing surgeries performed at CSU-ACC, the 1-year local recurrence-free rate determined by Kaplan-Meier life table analysis is over 76% with 60% alive at 1 year.[195] Local disease control was improved with certain treatments such as pretreatment with moderate doses of radiation and intra-arterial cisplatin or local implantation of polymer-cisplatin. The percentage of tumor necrosis has been shown to be predictive of outcome.[220]

In two case series, 40% and 47.5% of dogs, respectively, developed allograft infections.[195,216] The majority had their infections adequately controlled with systemic antibiotics with or without local antibiotics (antibiotic-impregnated polymethyl methacrylate beads).[203] Many of these dogs continued to have evidence of infection; however, their function was not severely affected. In severe and uncontrolled infections, allografts had to be removed and a small number of dogs required amputation. An unexpected finding has been that dogs with allograft infections experienced a statistically significant prolongation of overall survival times compared to dogs with limb sparing without infected allografts.[221] The reason for this is unclear but could be related to activation of immune effector cells and a response to cytokines, such as interleukins or tumor necrosis factor, elaborated in the face of chronic bacterial infection.

Surgery for nonappendicular and less common appendicular sites of OS

Certain primary bone tumors of the pelvis can be removed by techniques of hemipelvectomy and, although these surgeries are difficult, function and cosmetic outcome have been excellent (see Figure 23-6).[222] Bone tumors originating in proximal sites of the scapula can be successfully removed by partial scapulectomy (Figure 23-10).[171,223] Dogs function well with partial scapulectomy; however, gait abnormalities may occur after complete scapulectomy by disarticulation at the scapulohumeral joint. Aggressive postoperative physical therapy is important in these cases to regain normal function. Metatarsal and metacarpal locations can be treated with local resections or amputation.[31] Experience at our institution would support removal of a single bone or the central two bones in most dogs with good functional outcome. In small dogs, removal of the medial or lateral two bones can result in normal function. Resection to this extent has not been attempted in larger dogs.

Mandibulectomy and maxillectomy are appropriate surgeries for primary bone tumors of oral sites.[157-161,224-226] In a survey of owners of dogs undergoing partial mandibulectomy or maxillectomy, 85% were satisfied with the outcome despite 44% citing difficulty in eating as a complication.[227] Tumors of periorbital sites can be removed by orbitectomy.[228] Rib tumors can be removed by thoracic wall resection and the defect reconstructed with polypropylene mesh* with plastic plates† for large defects or by muscle flap techniques.[166-168] Diaphragmatic advancement can be used for caudally located defects. Small primary tumors of the ulna can be removed by partial ulnectomy, and reconstruction is rarely needed.[229] Vertebral OS sites are the most difficult with respect to adequately treating local disease. Techniques of complete vertebrectomy are not well established in veterinary medicine, and surgery often is an attempt to decompress dogs with neurologic deficits or intractable pain and to obtain a diagnosis.[173] Present recommendations are to perform surgery in cases that require decompression (with or without stabilization) and institute radiation therapy (discussed later) and chemotherapy.

Radiation

The combination of external beam radiation therapy and limb sparing has been described previously.[193,194] It appears that radiation therapy can cause considerable necrosis of primary OS in dogs. In this manner, radiation is used in an effort to downstage the primary tumor to improve the success of local disease control following removal. At present, the role of radiation therapy used to replace surgery, with or without systemic chemotherapy, is unclear. Currently, the role of radiation therapy in dogs with appendicular OS is primarily reserved for palliation of bone pain (see Palliative Treatment: Primary and Metastatic Bone Pain, presented later in this chapter). As a primary therapy, a median survival time of 209 days was reported in 14 dogs with appendicular OS treated with fractionated high-dose radiation (median dose of 57 Gy) to their primary tumor and systemic chemotherapy for micrometastasis.[230] Similar results are seen in people with extremity OS treated with high-dose radiation

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*Marlex mesh, CR Bard Inc., Billerica, MD.
†Lubra plates, Fort Collins, CO.

with and without surgical stabilization.[231] Radiation therapy likely plays a role in the treatment of OS of vertebrae. In a series of 14 dogs with vertebral OS treated between 1986 to 1995, 12 had surgery to decompress the spinal cord, 7 were treated with OPLA-Pt implanted in a distant intramuscular site, and 11 were given intravenous cisplatin. Nine dogs were treated with fractionated external beam radiation therapy. All dogs had surgery, radiation therapy, or both while no dog was treated with chemotherapy alone. Four dogs improved neurologically, four dogs worsened, and six dogs remained the same. The median survival of 135 days after treatment was relatively short.[173] Local disease recurrence rather than metastasis was the usual cause of death.

Stereotactic radiosurgery or intensity modulated radiation therapy (IMRT)

Stereotactic radiosurgery (gamma knife therapy) has been performed as a means of limb-salvage surgery in 11 dogs.[232] Dogs were treated using a frameless stereotactic radiosurgery system adapted from a system developed for the treatment of intracranial tumors in humans. In some cases, carboplatin was used immediately prior to treatment for its radiosensitizing potential in addition to its conventional cytotoxic qualities. In total, six dogs received adjuvant chemotherapy, and five dogs received radiosurgery alone. Five dogs developed pathologic fractures, and one dog developed infection. Acute effects to the skin were mild to moderate in most dogs. Impressively, overall median survival was 363 days in this series, albeit the numbers are still small. Advantages of this technique include the normal tissue-sparing effects that stereotactic radiation potentially provides and the ability to avoid surgery. Disadvantages are that the technique involves equipment that is not typically available to veterinarians. These results suggest that that stereotactic radiotherapy may provide a viable limb-sparing alternative, but further study is needed.

IMRT is a means of precisely delivering radiation therapy to tumors while conformally avoiding normal tissues. A form of IMRT, tomotherapy, utilizes a marriage of a CT scanner and a linear accelerator and has been investigated in dogs with OS and dogs with nasal tumors.[233] While such techniques are investigational in dogs at present, they would allow delivery of significant doses of radiation therapy that theoretically could be locally curative while sparing normal structures.

A   B

Figure 23-10

A, Preoperative radiograph (scapula technique) of an osteosarcoma of the proximal scapula. **B,** Specimen radiograph of the case in **A** after partial scapulectomy.

Radioisotopes

The bone-seeking radioisotope, [153]samarium ethylenedi-amine-tetramethylene phosphonate (samarium), has been used to treat osteosarcoma in dogs and humans. In high doses, samarium has been shown to locally deliver 20 to 200 Gy of radiation to normal bone and osteosarcoma tumors, respectively.[234] The efficacy of samarium in canine OS patients has been previously reported.[235,236,] Studies on samarium's efficacy for OS in dogs indicate that tumor doses equivalent to 20 Gy may be deposited in canine osteosarcomas using low to moderate doses of samarium, and the ratio between tumor dose and dose to surrounding tissues is favorable. The treatment provides pain relief in canine patients and, in some cases, tumor growth delay but is not curative.[236,237] In both normal beagle dogs and in tumor-bearing dogs, [153] Sm-EDTMP caused transient bone marrow depression (for approximately 4 weeks) of all cell lines.

Adjuvant Systemic Treatment for Dogs with OS

In general, dogs with osteosarcoma ultimately die of metastatic disease distant to the site of their primary tumor. That is, afflicted dogs do not die from a lack of adequate surgical therapy for the primary site; rather, they die as a result of inadequate adjuvant medical therapy for micro and macro-metastatic disease. This is not meant to trivialize the important work being done in both veterinary and physician-based oncology to improve and refine limb-sparing techniques. However, overall improvements in survival await advances in systemic therapy.

While the advent of the platinum class of chemotherapeutics has quadrupled median survival times in dogs receiving adequate primary site control, approximately 80% of dogs eventually die of distant disease. Much remains to be achieved regarding the prolongation of disease-free survival in dogs with OS and novel chemotherapeutics, drug delivery techniques, targeted molecular, and immunomodulatory therapies are the subject of intense research in the medical community. Current and promising future adjuvant therapies are discussed in the following section.

Chemotherapy

Table 23-3 lists commonly reported adjuvant chemotherapy protocols and outcomes for dogs following amputation. It is important to note that few of these protocols include large numbers of dogs and fewer compare treatment protocols in a randomized prospective fashion. Therefore, evaluations of efficacy between

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TABLE 23-3 **Commonly Used Adjuvant Chemotherapy Agents and the Survival Outcome for Dogs with Osteosarcoma Where Amputation Has Been Performed[a]**

Drug	Dose Regime and Number of Dogs	Disease Free	Survival	Comments
Cisplatin[14]	70 mg/m² IV on two occasions, every 21 days n = 26	Median 177–226 days	38%–43% at 1 year, 16%–18% at 2 years, median 262–282 days	No significant difference between survival data for dogs given cisplatin before amputation compared to those treated after amputation
Cisplatin[191] (some dogs treated with limb sparing)	60 mg/m² IV on one to six occasions, every 21 days n = 22	Not reported	45.5% at 1 year, 20.9 at 2 years, median 325 days	Apparent increase in treatment failures due to bone metastases
Cisplatin[238]	40–50 mg/m² IV on two to six occasions, every 28 days n = 11	Median 165 days	Median 300 days	
Cisplatin[239]	50 mg/m² IV on two occasions 2 and 7 weeks after amputation n = 15	Not reported	30% at 1 year, median 290 days	

[a]Few of these protocols include large numbers of dogs, and fewer compare treatment protocols in a randomized prospective fashion. Therefore, evaluations of efficacy between the various protocols are subject to bias and should be compared with caution.

TABLE 23-3 Commonly Used Adjuvant Chemotherapy Agents and the Survival Outcome for Dogs with Osteosarcoma Where Amputation Has Been Performed—cont'd

Drug	Dose Regime and Number of Dogs	Disease Free	Survival	Comments
Cisplatin[240]	50 mg/m² IV on up to nine occasions, every 28 days n = 16	Not reported	62% at 1 year median 413 days	Trend for dogs receiving higher cumulative doses of cisplatin to have longer survival times
Carboplatin[176]	300 mg/m² on four occasions every 21 days n = 48	Median 257 days	35.4% at 1 year median 321 days	Maximum tolerated cumulative dose has not been described for dogs
Doxorubicin[249]	30 mg/m² on five occasions every 2 weeks n = 35	Not reported	50.5% at 1 year and 9.7% at 2 years median 366 days	Percentage necrosis of tumor predicted survival
Doxorubicin and cisplatin alternating sequentially[264]	Doxorubicin at 30 mg/m² IV on day 1 and cisplatin at 60 mg/m² IV on day 21 cycle repeated once in 21 days n = 19	Median 210 days	37% at 1 year median 300 days	No significant difference was found between survival data from this study and survival data from a single agent cisplatin study[14]
Doxorubicin and cisplatin concurrent[262]	Doxorubicin at 15–20 mg/m² followed in 2 hours by cisplatin at 60 mg/m² n = 102	Not reported	48% at 1 year 28% at 2 years median 345 days	Twenty-two cases of dose reduction; no difference in postoperative versus preoperative chemotherapy
Doxorubicin and cisplatin concurrent[261]	Cisplatin at 50 mg/m² day 1 and doxorubicin at 15 mg/m² day 2 n = 14	Median 470 days	Median 540 days	Small sample size; another trial looking at a similar protocol with higher cisplatin dosing (60 mg/m²) and doxorubicin (25 mg/m²) was associated with unacceptable toxicity
Doxorubicin and carboplatin alternating sequentially[265]	Carboplatin (300 mg/m²) on day 1 and doxorubicin (30 mg/m²) on day 21, alternating at 3-week intervals for three cycles (six total treatments) n = 32	Median 227 days	Median 320 days 1-year survival = 48% 2-year survival = 18%	Includes both amputation and limb-salvage therapies for the primary
Doxorubicin and carboplatin concurrent[266]	Carboplatin (175 mg/m²) on day 1 and doxorubicin (15 mg/m²) on day 2, every 3 weeks, four treatments n = 24	Median = 195 days	Median = 235 days	The combination at these dosages was well tolerated in most cases
Lobaplatin[255]	Lobaplatin (35 mg/m²) every 3 weeks for four treatments	1-year disease free survival rate of 21.8%	1-year overall survival rate of 31.8%	No need for diuresis with this platinum analogue
OPLA-Pt[267]	80 mg/m² implanted at the time of amputation n = 37	Median 256 days	41.2% at 1 year median 278 days	New trials ongoing with an injectable polymer containing cisplatin

the various protocols are subject to bias and should be compared with caution.

Cisplatin

Cisplatin has been demonstrated to improve survival in dogs with OS after amputation, and it (or its analogue carboplatin, discussed later) are currently the standard of care for OS in dogs (see Table 23-3).[14,191,238-240] The ideal timing of cisplatin delivery, in relation to amputation, is currently unknown. In a report of 36 dogs with appendicular OS treated with cisplatin and amputation,[14] 17 dogs (group 1) were treated with two doses of IV cisplatin 21 days apart, beginning on average 18 days after amputation, and 19 dogs (group 2) were treated at diagnosis (preamputation) and received a second dose 21 days later, immediately after amputation. The median survival for group 1 (262 days; 1- and 2-year survival rates of 38% and 18%) was not significantly different from that of group 2 dogs (282 days; 1- and 2-year survival rates of 43% and 16%). There is a large body of evidence, however, reported in clinical studies of human OS and laboratory studies with rodents that supports perioperative use of chemotherapy, so called "neoadjuvant" or "primary" chemotherapy.[241-245] It seems reasonable to recommend the earliest possible administration of chemotherapy, which is usually at the time of amputation. However, no data exist to support a significant difference in survival for dogs treated preoperatively, immediately postoperatively or after a delay of up to 14 days postoperatively. Most current protocols involve four treatments of cisplatin, when it is used as a single agent; however, definitive studies in the dog have not been done to determine the most efficacious number of treatments. The recommended dose for cisplatin is 70 mg/m^2 body surface area, given intravenously every 3 weeks. In one study, a single dose of 90 mg/m^2 was given to 12 dogs concurrent with hypertonic saline, and there was no apparent nephrotoxicity in the short term.[246] However, myelosuppression and renal toxicity become serious and life-threatening complications occurred as the dose of cisplatin is increased. Due to the potential for nephrotoxicity, normal baseline renal function is, therefore, essential, and diuresis protocols must be performed concurrent with treatment. The diuresis protocol used most commonly[247] involves a 4-hour pretreatment diuresis with 18.3 ml/kg/hr of 0.9% NaCl, followed by a 20-minute infusion of cisplatin diluted in a volume of 0.9% NaCl that will allow the same (18.3 ml/kg/hr) fluid infusion rate as the prediuresis. The cisplatin infusion is then followed by an additional 2 hours of diuresis with 18.3 ml/kg/hr of 0.9% NaCl. The incorporation of cisplatin into sterically stabilized liposomes allows an increased dose (350 mg/m^2) to be safely given to canine patients without the necessity for diuresis; however, this formulation failed to improve patient survival over standard chemotherapy dosing in one study.[180] While no predictive pretreatment sensitivity assays are available to

determine if a particular dog's OS will be responsive to cisplatin chemotherapy, one study investigated the predictive ability of lymphocyte micronuclei frequency following cisplatin therapy.[248] Dogs having a micronuclear frequency of greater than 10% had median remission and survival times of 68.3 and 79.0 weeks, respectively, whereas those dogs with a micronuclear frequency of less than 10% had median remission and survival times of 14.1 and 17.9 weeks, respectively. In children with OS, chemotherapy is usually given in the neoadjuvant setting prior to definitive surgery and then assessment of percentage of tumor necrosis determined at the time of surgery. This "in situ" tumor sensitivity test is then used to direct subsequent chemotherapy after surgery based on the degree of necrosis occurring.[241-245] If the percentage of tumor necrosis is low, the response is considered inadequate and the postoperative chemotherapeutic regimen is often modified. The percentage of tumor necrosis was evaluated in resected specimens from dogs with OS treated with various preoperative regimes.[220] The mean percentage of tumor necrosis for untreated tumors (n = 94) 27%, radiation therapy alone (n = 23) 82%, two doses of intra-arterial (IA) cisplatin at 70 mg/m^2/dose (n = 14) 45%, two doses of IV cisplatin at 70 mg/m^2/dose (n = 6) 24%, two doses of IA cisplatin at 70 mg/m^2/dose and radiation therapy (n = 45) 82%, and 10 doses of IV cisplatin at 10 mg/m^2/dose and radiation therapy (n = 8) 78%. There was no significant difference between the percentage of tumor necrosis in untreated osteosarcoma compared to those receiving IV cisplatin alone, but a significant increase in the percentage of tumor necrosis was present in all other groups. The percentage of tumor necrosis was strongly correlated with local tumor control after limb sparing, as 91% of dogs with >90% tumor necrosis had local control, while 78% of dogs with 80% to 89% tumor necrosis had local tumor control and only 30% of dogs with < 79% tumor necrosis had local control. Since those dogs with radiation as part of their preoperative treatments had a higher mean percentage of tumor necrosis, radiation in combination with chemotherapy may still play a role in the local preoperative management of osteosarcoma. The percentage of tumor necrosis in resected primary tumors from dogs with OS treated with intravenous doxorubicin ranged from 0 to 87% (mean, 24.9%) in one report.[249] Interestingly, there was a significant direct correlation between survival time and the percentage of necrosis in that study.

Carboplatin and lobaplatin

Carboplatin* is a second-generation platinum compound that is less nephrotoxic than cisplatin with apparently similar antitumor effects.[176,250-254] In a multi-institutional study of 48 dogs with appendicular OS treated with amputation and up to four doses of carboplatin the

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*Paraplatin, Bristol-Meyers, Squibb Laboratories, Evansville, IN.

median disease-free interval was 257 days, the median survival was 321 days, and 35.4% of dogs were alive at 1 year (see Table 23-2).[176] One of the advantages with carboplatin is that it can be given without the saline diuresis necessary for cisplatin administration as it is not nephrotoxic like its predecessor, cisplatin. A caveat to carboplatin use, however, is that while it is not nephrotoxic, it is almost exclusively cleared by renal means, and if preexisting renal function is compromised, significant myelosuppression can occur as drug clearance is diminished and marrow is exposed to drug for longer periods of time. In human patients, carboplatin dose is determined based on knowledge of creatinine clearance of the individual patient, not body weight or surface area. In veterinary medicine, no accurate creatinine clearance considering formulas have been determined, and carboplatin should be avoided in dogs with renal compromise until a safe dosing formula is developed. The drug can be given at amputation and in subsequent 21-day cycles provided peripheral blood counts and renal function remain adequate. The dose recommended for use in dogs is 300 mg/m^2 administered every 3 weeks for four treatments; however, the maximum tolerated cumulative dose has not been described; most current protocols involve four cycles when used as a single agent. Lobaplatin, a third-generation platinum compound, has been investigated in dogs, and in an adjuvant study of 28 dogs treated with 35 mg/m^2 q 3 weeks for four treatments, the drug was well tolerated; however, those patients had a 32% 1-year survival, less than that seen in historical sets of dogs treated with carboplatin or cisplatin.[255]

Doxorubicin

Doxorubicin is also an effective agent for adjuvant chemotherapy in dogs with OS; however, it is not as efficacious as the platinum agents when used as a single agent. A poor response to adjuvant doxorubicin as a single agent was determined by one report of 16 dogs with OS.[256] In that study, doxorubicin was given intravenously at a dosage of 30 mg/m^2 every 3 weeks, beginning 3 weeks after surgery. In a more recent study, doxorubicin was given at the same dosage but every 2 weeks for five treatments to 35 dogs with appendicular OS, and surgical excision was performed either 13 days after the second or third treatment with the subsequent treatment given on the day after surgery.[249] The 1- and 2-year survival rates were 50.5% and 9.7%, respectively. No difference was found between dogs treated prior to or immediately following amputation. Why the 2-year survival is less than that for cisplatin is unclear. Although the increased dose intensity (administered at 2-week intervals) is suspected to be the reason for the improved efficacy of doxorubicin when compared to previous studies (administered at 3-week intervals), further evaluation of the importance of scheduling is warranted. The CSU-ACC experience with single-agent doxorubicin in a larger group of dogs with OS (N = >200; CSU database) results in median survivals of approximately 7.5 months. Concern has been raised over the potential induction of chemotherapy resistance for OS treated with doxorubicin, as this drug has been shown to increase p-glycoprotein related resistance in other tumors. Although p-glycoprotein (PGP) has been shown to be upregulated in OS cell lines exposed to doxorubicin, one report did not show an increase in multiple drug resistance related protein (MRP) in these same lines.[257] The radionucleotide *Tc99m*-sestamibi (MIBI, Sestamibi) has been shown to be predictive of MDR as decreased uptake into tumors is correlated with increased activity of the p-glycoprotein pump.[258,259] In a another study, use of the MDR reversal agent Valspodar (PSC 933) with doxorubicin in canine OS patients allowed a dose reduction in the doxorubicin with maintenance of therapeutic plasma levels of drug.[260] This would support an MDR reversal effect clinically in these patients although the clinical relevance of this is currently unknown.

Other adjuvant chemotherapy protocols

Several other adjuvant chemotherapy protocols have been reported in dogs with OS and are also presented in Table 23-3. It would seem reasonable that combinations of drugs shown to be efficacious alone could further improve survival times. Several nonrandomized single-arm studies have evaluated combinations of platinum agents (either cisplatin or carboplatin) and doxorubicin either given concurrently or alternating sequentially. A small study evaluated the combination of doxorubicin and cisplatin concomitantly. Cisplatin was given beginning the day of surgery at 50 mg/m^2, followed by doxorubicin at 10 mg/m^2 the following day. Toxicity was tolerable and the median survival of 16 dogs was approaching 18 months.[261] In a larger study, doxorubicin was given at 15 to 20 mg/m^2, followed 2 hours later by cisplatin, given at 60 mg/m^2. Similar toxicity was seen; however, the median survival for those 102 dogs was slightly greater than 11 months, apparently no better than historical comparisons to dogs receiving single-agent cisplatin.[262] In another study, unacceptable toxicity was seen, when the dose of doxorubicin and cisplatin in the combination was increased to 25 mg/m^2 and 60 mg/m^2, respectively.[263] Toxicity was also magnified when given 2 days following amputation compared to administration 14 days postoperatively. Rather than using doxorubicin and cisplatin concurrently at lower doses, these two drugs have also been given at full doses in 3-week sequential intervals. In one such report, 19 dogs were given alternating doses of doxorubicin (30 mg/m^2) and cisplaltin (60 mg/m^2).[264] Carboplatin has also been substituted for cisplatin in a similar sequential combination protocol; a disease-free interval (DFI) of 227 days and an overall survival of 320 days was reported for dogs treated with three alternating doses each of

carboplatin and doxorubicin at 21-day intervals.[265] In another study, concurrent carboplatin (175 mg/ m² IV, day 1) and doxorubicin (15 mg/m² IV, day 2) given on a 21-day cycle for a maximum of four cycles was shown to be well tolerated; however, median disease-free interval was 195 days, and median survival was 235 days showing minimal or no advantage over single agent protocols.[266] These results also do not support increased response outcome for combination therapy when compared to single-agent platinum compounds.

Investigational Systemic Adjuvant Therapies

Polymer chemotherapy delivery

A previously discussed drug delivery system, OPLA-Pt, releases a high dose of chemotherapy into a surgical wound and causes slow release of relatively low concentrations of chemotherapy systemically. When OPLA-Pt was implanted in normal dogs no systemic toxicity and no impedance of cortical allograft healing was identified at doses of up to 80.6 mg/m².[267] Serum pharmacology data revealed approximately a 30-fold increase in area under the curve (AUC) for systemic platinum exposure compared to a similar dose of intravenous cisplatin. Local wound concentrations of platinum are up to 50 times that achievable from a single intravenous dose. This system has also been investigated for the control of microscopic distant disease. Thirty-nine dogs with stage IIB appendicular OS were treated with amputation and one dose of OPLA-Pt implanted (median 86 mg/m²) in the muscles of the amputation stump at the time of surgery.[267] The median survival was 240 days, and the 1-year survival rate was 41.2%. This is equivalent to the improvement in survival probability when two intravenous doses of cisplatin are used with amputation. When the effect of drug AUC on survival was evaluated in this same group of dogs, an AUC of greater than 7 ug/ml/min had a significantly longer survival time than those below 7 (Figure 23-11). This finding supports adjuvant treatment of OS using sustained release drug delivery systems. Another study did not demonstrate an advantage to giving a second dose of OPLA-Pt (4 weeks after the first) after amputation.[268] To date, local tissue toxicity to the implant system is the primary complication seen. The authors are currently evaluating another form of injectable polylactic acid polymer system (Atrigel)* for sustained release of cisplatin. Neither OPLA-Pt nor Atriplat are currently available commercially.

Immunotherapy

Most immunotherapy trials for dogs with OS involve the immune stimulant liposome-encapsulated muramyl

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*Atrigel[R], Atrix Laboratories, Fort Collins, CO.
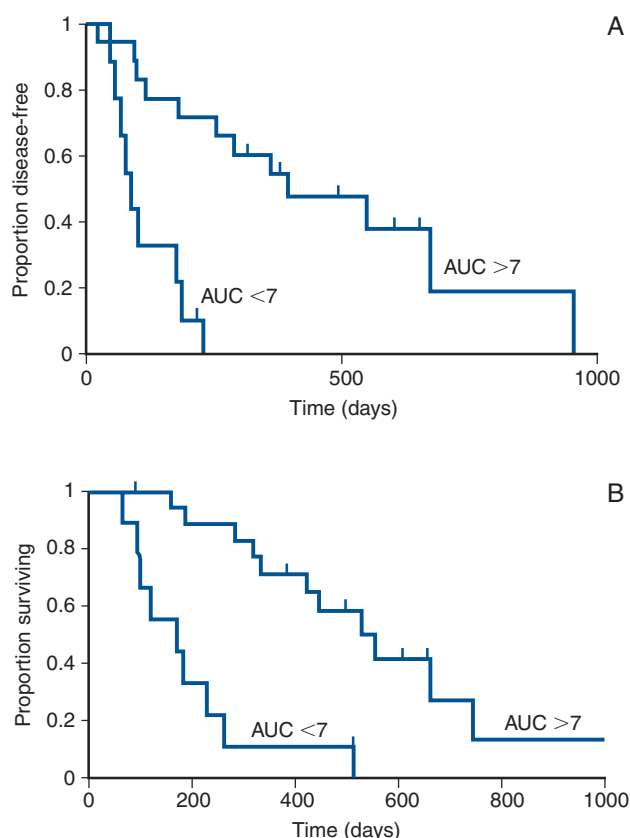
Figure 23-11

A, Kaplan-Meier curve showing a significant difference in disease-free interval between dogs with area under the platinum concentration versus time curve (AUC) less than 7 ug/ml/day (n = 9) and dogs with AUC greater than 7 (n = 19). **B,** Kaplan-Meier curve showing significant difference in survival between dogs with area under the platinum concentration versus time curve (AUC) less than 7 ug/ml/day (n = 9) and dogs with AUC greater than 7 (n = 19). *(Reprinted with permission from S. J. Withrow, R. C. Straw, J. H. Brekke, et al., "Slow release adjuvant cisplatin for the treatment of metastatic canine osteosarcoma," Eur J Musculoskel Res 4:108, 1995.)*

tripeptide-phosphatidylethanolamine (L-MTP-PE). L-MTP-PE is a lipophilic derivative of muramyl dipeptide, which is a synthetic analogue of a fragment of *Mycobacterium* cell wall. L-MTP-PE has been shown to activate canine alveolar macrophages and enhance their cytotoxicity of OS cells *in vitro*.[269] The combination of L-MTP-PE and doxorubicin was also shown to synergistically enhance the antitumor activity of canine alveolar macrophages *in vitro* and result in increased levels of circulating tumor necrosis factor and IL-2 in dogs with hemangiosarcoma.[270,271] Likewise, the combination of ifosfamide and L-MTP-PE resulted in increased mononuclear cell tumoricidal activity and cytokine

release *in vitro*, using human mononuclear effector cells.[272] In initial studies, dogs treated with amputation and intravenously administered L-MTP-PE experienced median survivals of 222 days, significantly greater than amputation alone.[273] In a follow-up study, dogs randomized to L-MTP-PE, *following* four doses of cisplatin (70 mg/m^2, q 3 weeks) had a median survival time of 432 days (14.5 months) versus 291 days (9.7 months) for those dogs receiving four doses of cisplatin and placebo liposomes.[274] In contrast, when L-MTP-PE was administered *concomitantly* with cisplatin, this advantage was lost. It is possible that concurrent use with cisplatin results in a negative interaction, or that cisplatin decreases cellular immunity. The Children's Oncology Group (COG) and the Pediatric Oncology Group (POG) coordinated a national trial in children using L-MTP-PE in combination with chemotherapy in children following surgery. Preliminary analyses indicated that L-MTP-PE combined with doxorubicin, cisplatin, high-dose methotrexate, and ifosfamide was superior to the same chemotherapy given alone; however, due to the factorial study design and issues with entry criteria, questions remain on efficacy, and a follow-up trial in children is currently ongoing.[275,276] Several other immunotherapy approaches have been investigated in dogs with OS in the setting of gross metastatic disease (see the subsequent section).

Molecular Targeted Therapies for Dogs with OS

There is a growing body of investigation into small molecule, targeted, or static therapies for the treatment of OS micrometastases. It has long been theorized that growth hormone (GH) or insulin-like growth factor (IGF-1) may play a role in OS development in children, as both are present in high levels during the decade of risk associated with growth. To investigate the possibility of targeting this potential growth-stimulating process, a randomized clinical trial in pet dogs with OS was undertaken where IGF-1 was suppressed with a long-acting analogue of somatostatin (OncoLAR, octreotide pamoate long-acting release).[277] A combination of OncoLAR and carboplatin in OS dogs following amputation did suppress IGF-1 levels by approximately half; however, this did not translate into enhanced survival times. It was theorized that the extent and duration of IGF-1 suppression might have been insufficient in this protocol.

A number of reports have evaluated the potential therapeutic effects of bisphosphonates for canine OS both *in vitro* and *in vivo*. Both cytotoxic and cytostatic effects were seen in canine OS cell lines exposed to pamidronate, alendronate, and zoledronate *in vitro* in a dose-dependent manner.[104-106] Additionally, surrogate biological activity was shown *in vivo* following pamidronate administration in dogs with gross primary OS as assessed by reduction in urinary N-telopeptide excretion and enhanced bone mineral density (dual-energy x-ray absorptiometry).[278] Whether the *in vitro* and surrogate *in vivo* effects translate into survival advantages in dogs with OS awaits investigation with controlled clinical trials.

In a prospective, randomized clinical trial using dogs with naturally occurring appendicular OS, the therapeutic potential of an inhibitor of protein-kinase-C (a potent stimulator of tumor cell proliferation) dexniguldipine was evaluated.[279] Dexniguldipine- and cisplatin-treated dogs had longer median remission duration and survival time than untreated dogs; however, dexniguldipine-treated dogs had a shorter survival time than cisplatin-treated dogs. The combination of dexniguldipine and chemotherapy has not been investigated in dogs.

Several other agents have been investigated in preclinical models and await clinical trial investigations. For example, COX-2 has been shown to be overexpressed in human and canine OS tumors, making it an attractive target for therapeutic intervention.[95] Indeed, meloxicam has been shown to have antineoplastic effects on canine OS cells *in vitro*.[280] Additionally, thalidomide, a drug that is gaining a modest resurgence in physician-based oncology as a potential antiangiogenesis agent, was found to potentially interfere with the ability of canine OS cells to metastasis in a preclinical mouse xenotransplantation model.[281] Finally, reestablishing wild-type p53 (Chapter 2) expression in canine OS cell lines affects their growth characteristics.[78] All of these agents will require further investigations to determine if preclinical activity translates to antitumor activity in pet dogs with OS.

Treating Gross Metastatic Disease

Surgery for gross metastatic disease

Resection of pulmonary metastasis from OS or other solid tumors has been reported in people.[282] One report of 36 dogs treated with pulmonary metastasectomy for OS exists.[283] Lesions located subpleurally were gently lifted from the lung parenchyma by thumb forceps and a single pursestring of 2-0 or 3-0 polygalactin 910* suture was tied around the base of normal tissue. Larger lesions located deeper in the lung parenchyma were removed by complete or partial lobectomy using surgical staples.† No chemotherapy was given after these surgeries. Although the initial treatments varied between dogs, the median survival time of the entire group was 487 days. The median survival after pulmonary metastasectomy was

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*Vicryl, Ethicon, Sommerville, NJ.
†TA30 or TA55, United States Surgical Corp., New York, NY.

176 days (range 20 to 1495 days). The criteria established for case selection for pulmonary metastasectomy in order to maximize the probability of long survival periods are (1) primary tumor in complete remission, preferably for a long relapse-free interval (>300 days); (2) one or two nodules visible on plain thoracic radiographs; (3) cancer only found in the lung (negative bone scan); and perhaps (4) long doubling time (>30 days) with no new visible lesions within this time. Surgical intervention for other metastatic sites has been attempted in a few cases at the authors' institution with subjective response; however, the number of patients treated are too small to make conclusions or base recommendations on at this time.

Chemotherapy for gross (macroscopic) OS

Chemotherapy has also been used in a nonadjuvant setting in dogs with gross, measurable OS. In this setting, response rates and the durability of response have been disappointing. In 45 dogs with gross metastatic OS treated with various types of single-agent chemotherapy (31 with cisplatin, 11 with doxorubicin, and 3 with mitoxantrone), only one achieved a response (partial) that lasted for 21 days, and the rest progressed.[284] One small study evaluated paclitaxel as primary therapy for a variety of malignancies including macroscopic OS.[285] In this trial, two of nine dogs with pulmonary metastatic osteosarcoma responded (>50% volume reduction). This is a difficult drug to give due to the allergic properties of the diluent; however, safe dose and administration techniques are now available and further evaluation of its efficacy, both as a single agent and in combination, may be warranted.

Investigational Therapies for Gross Metastatic Disease

Aerosol drug delivery

The development of aerosol technology for administration of chemotherapy may provide a novel approach for treatment of OS lung metastases. Aerosol delivery alters biodistribution in favor of pulmonary deposition.[286] This is in contrast to systemic delivery, where the drug is first diluted in the blood before it reaches the lung. In veterinary patients, measurable responses to inhalational taxol and doxorubicin have been observed for metastastic OS in pet dogs in small pilot studies.[287,288]

One study evaluated the efficacy of aerosol gemcitabine on the growth of primary and metastatic OS xenografts in mice.[289-291] Although the administration of gemcitabine intravenously or intraperitoneally had no effect, gemcitabine delivered by aerosol had a significant effect on lung metastases as well as upregulating Fas expression in the metastatic OS cell lines used in the study. The Fas/FasL death receptor pathway (see Chapter 2) has been identified as a key mediator of chemotherapy-induced apoptosis in several tumor types. Because Fas ligand is constitutively expressed by lung epithelium, agents that upregulate Fas expression delivered directly to the lung may have an advantage.

Immunotherapy for gross metastatic disease

Inhalational therapy using the potent immunomodulatory cytokine interleukin-2 (IL-2) has been investigated in normal dogs and pet dogs with metastatic OS.[292,293] Inhaled IL-2 resulted in enhanced activation of pulmonary immune effector cells. Additionally, complete regression of pulmonary OS lesions occurred in 2 dogs following inhalational IL-2 delivery.

A liposome-DNA complex encoding IL-2 has also shown potential promise for metastatic OS.[294] Preliminary work using this liposome-DNA complex improved survival in dogs with metastatic OS threefold over untreated (historical) controls. These preliminary investigations in the gross disease setting support further development, in particular as adjuvants in the micrometastatic disease setting and in combination with currently available therapies.

Palliative Treatment: Primary and Metastatic Bone Pain

Palliative radiation therapy

Radiation therapy to palliate bone pain has been investigated and extensively applied in veterinary oncology.[295-299] Using protocols ranging from two to four 8-Gy fractions, pain management is palliated in 74% to 92% of dogs for medians ranging from 2 to 4 months. More specifically, a protocol of three 10-Gy fractions delivered over a 3-week period on days 0, 7, and 21, for a total dose of 30 Gy, has been described for palliative treatment of OS in dogs.[295] For distal extremity lesions, daily or every-other-day treatment with three 8-Gy fractions can be performed without marked radiation toxicity. Over 70% of dogs responded positively (improvement in clinical evidence of pain and lameness) for an average of 2 months in one study using either 3, 10-Gy fractions (0,7,21 day schedule) or 2, 8-Gy fractions (0,7 day schedule).[296] In that study and an older report,[299] addition of cisplatin chemotherapy appeared to have a benefit in response rate; however, larger randomized investigations are warranted to document this advantage. One study failed to demonstrate a difference in response for either three 8-Gy or four 6-Gy fractionation schemes using electron therapy.[297] In another study, four 8-Gy fractions were well tolerated and resulted in a 90% response rate in 24 patients.[298] Adding additional fractions were beyond the initial three after clinical signs have returned has been successful for further temporary pain relief in a few dogs; however, the potential for marked acute radiation toxicity increases. Palliative radiation is a useful and effective

treatment option for dogs with stage III disease at presentation (that is, distant metastasis to lung, for example) or where the owner does not want to pursue attempts at long-term local control. In physician-based oncology, investigations have shown single large fractions (e.g., 8 to 15 Gy) are as effective as multiple smaller fractions for palliating bone pain.[300-302] A caveat to this is that larger single fraction doses were associated with a slightly higher pathologic bone fracture rate. The authors have anecdotally observed similar positive results with a single large fraction in dogs with OS, and while this is more cost and time effective, observation of a larger group of treated dogs would be necessary to confirm this finding.

Radiopharmaceuticals (such as strontium and samarium) have also been used to palliate pain from metastatic bone cancer in people.[303-305] As mentioned earlier, samarium-153-ethylenediamine-tetramethylene-phosphonic acid (^{153}Sm-EDTMP) is a radiopharmaceutical that has been used to treat metastatic and primary bone tumors in dogs.[305]

Pharmacologic palliation

For short-term pain control, a nonsteroidal anti-inflammatory drug (piroxicam, carprofen, meloxicam, etc.) may be given, dependent on control of clinical signs. This appears to give temporary pain relief to most dogs with OS lesions. Dogs must be carefully monitored for signs of gastrointestinal toxicity and the drug withdrawn if these signs occur. Corticosteroids must not be administered at the same time because this combination can predispose to the development of gastric or duodenal ulceration. It is also not advisable to give corticosteroids or piroxicam concurrently with cisplatin or in dogs with decreased renal function. There has been some work that suggests cyclooxygenase inhibitors such as piroxicam may have some direct or indirect antitumor effects.[306] Other choices, including acetaminophen/codeine combinations, and other opiates, are discussed in Chapter 16, Section A.

Bisphosphonates, diphosphonates, and other compounds have been used in people to control bone loss and subsequent pain due to metastatic lesions.[307,308] Two reports exist on the use of bisphosphonates to palliate dogs with appendicular OS.[278,309] In one report, 4 of 10 dogs treated appeared to have modest subjective improvement in pain control following pamidronate therapy (1.0 mg/kg, given as a 2-hour constant rate infusion in 250 cc 0.9% NaCl).[278] In the other report, daily oral alendronate was given to two dogs with OS, one of the maxilla and the other of the tibia.[309] The tibial OS was stabilized with an external fixator. Both dogs remained comfortable and survived 10 and 12 months, respectively.

Comparative Aspects

Animal models for the study of human diseases are important to our understanding of the mechanism and etiology of disease and for the development and refinement of therapeutic strategies. Spontaneously developing diseases in animal populations are particularly useful for study.[310] Canine osteosarcoma has many similarities to human osteosarcoma and can serve well as a valuable comparative model for study (Table 23-4).[7] Osteosarcoma is more common in dogs than in humans; therefore case accrual is more rapid. Since disease progression is more rapid in dogs than in humans, results of treatment protocols can be reported earlier than would those of similar trials in humans. Research costs are less for clinical trials in dogs compared to those in human clinical trials, and, from an animal welfare standpoint, no disease is induced and dogs with cancer can be helped through the course of the research.

Osteosarcoma is an uncommon cancer of humans affecting mainly children in their second decade of life, and it remains a very serious, aggressive solid tumor. Fortunately there has been a great improvement in survival rates with the use of established multidrug adjuvant protocols. The long-term survival rate for human OS is presently 60%, which contrasts to the 20% expected 5-year survival rates of the early 1980s. A retrospective study of 648 human OS patients reported a mean survival of 3 years; however, this represented all cases at one institution since the 1970s and therefore improvements over time were not presented.[311] Factors that negatively affected outcome included older age, advanced local or systemic stage, axial location, larger size, and a lower percentage of necrosis following neoadjuvant treatment. The type of surgery (amputation or limb sparing) did not impact outcome, supporting the need for advancement of systemic medical therapy to impact survival. Limb-sparing programs are becoming more common, and many survivors of OS retain functional, pain-free limbs. In two reports, one from the United States[312] and one from Italy,[313] aggressive neoadjuvant chemotherapy resulted in limb-spare success of 93.5% and 83%, respectively, and a projected 10-year survival of 93%.

Bone Surface Osteosarcoma

Osteosarcoma usually originates from elements within the medullary canal of bones (intraosseous-OS); however, there are forms of this cancer that originate from the outside surface of bones. Periosteal OS is a high-grade form of surface OS and seems to arise from the periosteal surface but has invasive characteristics seen radiographically.[314] There is cortical lysis with extension of the tumor into the bone and surrounding soft tissues. These tumors are histologically similar to intraosseous OS and have similar aggressive biological behavior. In contrast, parosteal OS, or juxtacortical OS, arises from the periosteal surface of bones but appears

TABLE 23-4   A Comparison of Canine and Human Osteosarcoma Characteristics

Variable	Dog	Human
Incidence in United States	>8000/year	1000/year
Mean age	7 years	14 years
Race/breed	Large or giant purebreds	None
Body weight	90% > 20 kg	Heavy
Site	77% long bones	90% long bones
	Metaphyseal	Metaphyseal
	Distal radius > proximal humerus	Distal femur > proximal tibia
	Distal femur > tibia	Proximal humerus
Etiology	Generally unknown	Generally unknown
Percentage clinically confined to the limb at presentation	80%–90%	80%–90%
Percentage histologically high grade	95%	85%–90%
DNA index	75% aneuploid	75% aneuploid
Molecular and genetic alterations	See Table 23-1	See Table 23-1
Prognostic indicators[114,118,178-180]	Alkaline phosphatase	Alkaline phosphatase
Metastatic rate without chemotherapy	90% before 1 year	80% before 2 years
Metastatic sites	Lung > bone > soft tissue	Lung > bone > soft tissue
Improved survival with chemotherapy	Yes	Yes
Regional lymph node metastasis	< 5%, negative prognostically	Poor prognosis

Modified with permission from S. J. Withrow, B. E. Powers, R. C. Straw, and R. M. Wilkins, "Comparative aspects of osteosarcoma; dog versus man," Clin Orthop Relat Res 270: 159–167, 1991.[7]

less aggressive than periosteal OS both radiographically and in terms of biologic behavior. Parosteal osteosarcomas are relatively uncommon and have a moderately well-circumscribed radiographic appearance. The tumors grow out from the periosteal side of a cortex and cortical lysis is usually very mild on radiographs. Histologically, these tumors look more benign compared to intraosseous or periosteal-osteosarcoma. These tumors contain well-differentiated cartilage, fibrous tissue, and bone with sparse regions of sarcoma cells adjacent to tumor osteoid. Histologic specimens must be evaluated carefully because it is often easy to miss the areas of tumor cells and misdiagnose the lesion as osteoma, chondroma, or reactive bone. These tumors generally do not invade the medullary canal and tend to grow out from the bone on broad pedicles. Diagnosis is based on typical histologic and radiographic findings.

Parosteal OS is usually slow growing but can induce pain at the local site. Metastases can occur but the prognosis for long-term survival is much better than for intraosseous osteosarcoma.[315,316] Control of parosteal OS can be achieved by en bloc resection of the tumor with the adjacent cortical bone. This has been reported for tumors of the zygomatic arch (Figure 23-12). If full thickness cortex needs to be removed for tumors on long bones, reconstruction may be performed using autogenous corticocancellous bone such as a rib, ileal crest, or allogeneic cortical bone. A report described a surface OS without cortical destruction (similar to parosteal) that had an aggressive histology and biologic behavior.[317]

OTHER PRIMARY BONE TUMORS OF DOGS

It can be difficult to distinguish chondroblastic osteosarcoma from chondrosarcoma and fibroblastic osteosarcoma from fibrosarcoma and telangiectatic osteosarcoma from hemangiosarcoma when only small amounts of biopsy tissue are evaluated.[141] This makes interpretation of older reports difficult in terms of trying to establish the true incidence of the different types of primary bone tumors. This also underscores the importance of evaluating the entire excised specimen to validate the preoperative biopsy. All too often a bone malignancy thought to be relatively low grade from preoperative biopsy is upgraded to a true OS once the histology of the surgical specimen is reviewed. This may change the prognosis and postsurgical treatment plan.

A   B
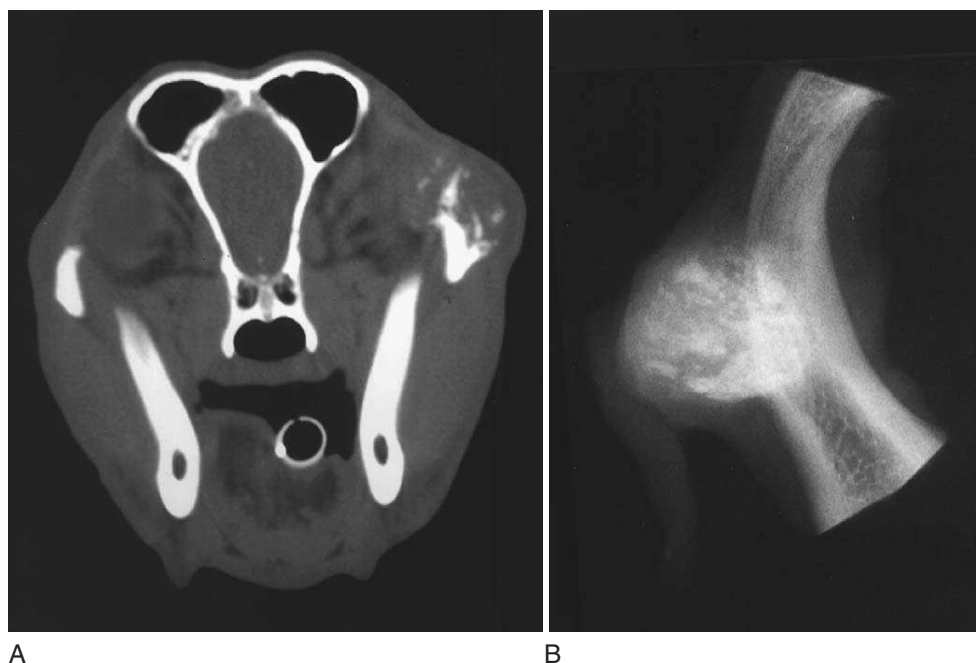
Figure 23-12

A, Computed tomographic scan of a low-grade parosteal osteosarcoma of the zygomatic arch in a dog. Low-grade primary bone tumors are often more radiodense and well circumscribed. **B,** Specimen radiograph of the case in **A** after zygomatic arch resection.

Primary bone tumors other than OS make up somewhere between 5% and 10% of bone malignancies in dogs. These tumors are chondrosarcomas, hemangiosarcomas, fibrosarcomas, lymphomas, and plasma cell tumors.

Chondrosarcoma

Chondrosarcoma (CS) is the second most common primary tumor of bone in humans and dogs and accounts for approximately 5% to 10% of all canine primary bone tumors.[2,3,5,318,319] Chondrosarcomas are characterized histologically by anaplastic cartilage cells that elaborate a cartilaginous matrix. There is a spectrum of degree of differentiation and maturation of the cells within and between each tumor. Histological grading systems have been devised.[320] The etiology is generally unknown although CS can arise in dogs with preexisting multiple cartilaginous exostosis.[321,322] In a clinicopathologic study of 97 dogs with CS, the mean age was 8.7 years (ranging from 1 to 15 years) and golden retrievers were at a higher risk of developing CS than any other breed.[323] There was no sex predilection, and 61% of the tumors occurred on flat bones. Chondrosarcoma can originate in the nasal cavity, ribs, long bones, pelvis, extraskeletal sites (such as the mammary gland, heart valves, aorta, larynx, trachea, lung, and omentum), vertebrae, facial bones, digits and os penis.[40,320,323-330] The nasal cavity is the most common site for canine chondrosarcoma.[320,321,323]

Chondrosarcoma is generally considered to be slow to metastasize. Tumor location rather than histological grade was prognostic in one study.[320] The reported median survival of dogs with nasal CS ranges from 210 days to 580 days with various treatments (radiation therapy, rhinotomy and radiation therapy, and rhinotomy alone; see Chapter 22, Section D).[323,331] Metastatic disease is not a reported feature of nasal CS in dogs. The reported median survival for dogs with CS of ribs varies widely.[27,166,318,320,332] Reports prior to 1992 contained few cases that were treated with intent to cure, but 15 dogs with rib CS treated with en bloc resection in a more recent study had a median survival of 1080 days.[168] The median survival for dogs with CS was 540 days in a study of five dogs treated with amputation alone.[323] Death was usually associated with metastatic disease. A reliable adjuvant chemotherapeutic agent is not known for canine chondrosarcoma. In people, CS is considered a local disease, with a moderate rate of metastasis, which can be predicted by histologic grade. Aggressive surgical resection often results in long-term tumor control.[333] While this tumor is generally considered resistant to standard radiation therapy, the authors have noted objective responses to coarse-fraction radiation protocols in a handful of cases where surgery was not an option.

Hemangiosarcoma

Primary hemangiosarcoma (HS) of bone is rare and accounts for less than 5% of all bone tumors (see Chapter 32, Section A). This disease generally affects middle-aged to older dogs and can occur in dogs of any size. This is a highly metastatic tumor, and virtually all dogs affected will develop measurable metastatic disease within 6 months of diagnosis. Metastases can be widely spread throughout various organs such as lungs, liver, spleen, heart, skeletal muscles, kidney, brain, and bones. Dogs can present with multiple lesions making it difficult to determine the site of primary disease. Histologically, HS is composed of highly anaplastic mesenchymal cells, which are precursors to vascular endothelium. The cells are arranged in chords separated by a collagenous background and may appear to be forming vascular channels or sinuses. Cellular pleomorphism and numerous mitotic figures are features of this highly malignant disease. There is profound bone lysis, and the malignant cells aggressively invade adjacent normal structures. The lesion, however, may be confused with telangiectatic osteosarcoma, especially if the diagnosis is based on small tissue samples. Often the dominant radiographic feature is lysis; however, HS does not have an unequivocally unique radiographic appearance, and diagnosis is based on histology.

If HS is diagnosed, the dog must be thoroughly staged with thoracic and abdominal films, bone survey radiography or bone scintigraphy, and ultrasonographic evaluation, particularly of the heart and abdominal organs. Right atrial HS may be present without clinical or radiographic signs of pericardial effusion. The prognosis is poor, and even dogs with HS clinically confined to one bony site have less than a 10% probability of surviving 1 year if the tumor can be completely excised. Cyclophosphamide, vincristine, and doxorubicin have been used in combination as an adjuvant protocol and the reported median survival of dogs with nonskeletal HS is 172 days.[334] In a patient population represented by a variety of primary tumor sites, doxorubicin as a single agent adjuvant seemed to be as effective as the combination of drugs, with a median survival time of 172 days in patients where all gross disease is surgically resected.[335] In a group of dogs with splenic hemangiosarcoma, L-MTP-PE resulted in a median survival of 277 days, compared to 143 days for dogs receiving empty liposomes.[271]

Fibrosarcoma

Primary fibrosarcoma (FS) is also a rare tumor of dogs and accounts for less than 5% of all primary bone tumors.[3] Unfortunately, the difficulty in distinguishing FS from fibroblastic OS histologically (especially from small tissue samples) renders study of this tumor difficult.

In one report, 11 dogs thought to have FS were reevaluated after complete resection and the histological diagnosis was changed to OS in 6 dogs.[336] Histologic characteristics of FS have been described as interwoven bundles of fibroblasts within a collagen matrix permeating cancellous and cortical bone but not associated with osteoid produced by the tumor cells. Host-derived new bone can be seen, however, especially at the periphery of the tumor.

Complete surgical resection of the primary lesion is recommended for dogs with FS clinically confined to the primary site. This treatment may be curative, although metastatic potential may be considerable. There is no good evidence that adjuvant chemotherapy is of any benefit in preventing metastatic disease. It has been postulated that primary FS of bone has a propensity to metastasize to such sites as heart, pericardium, skin, and bones rather than lung.[336]

Multilobular Osteochondrosarcoma

Multilobular osteochondrosarcoma (MLO) is an uncommon tumor that generally arises from the skull of dogs.[337-339] Many names have been used to describe this disease, including chondroma rodens and multilobular osteoma. These tumors have a characteristic appearance on radiographs, CT, and MRI: generally the borders of the tumor are sharply demarcated with limited lysis of adjacent bone, and there is a coarse granular mineral density throughout (Figure 23-13).[340,341] However, there is one report of an MLO of the vertebra that did not have radiographic abnormalities.[342] Histologically, these tumors are composed of multiple lobules each centered on a core of cartilaginous or bony matrix that is surrounded by a thin layer of spindle cells. A histologic grading system has been described.[337,338] These tumors have the potential to recur locally following incomplete resection, and metastasis can occur. In one report of 39 dogs, the median age of affected dogs was 8 years, the median weight was 29 kg, and there was no breed or sex predilection.[338] Slightly less than 50% of dogs had local tumor recurrence following resection at a median time of approximately 800 days. A little over half the dogs developed metastases after treatment; however, time to metastasis was prolonged with a median of 542 days. The median survival time was 800 days. Local tumor recurrence and metastasis after treatment appears to be predicted by histological grade and the ability to obtain histologically complete resection. Local tumor excision with histologically complete surgical margins appears to offer a good opportunity for long-term tumor control, especially for low-grade lesions. When metastatic lesions are identified by thoracic radiography, dogs may remain asymptomatic for their lung disease for up to 1 year or more. The role of chemotherapy and radiation therapy in the management of MLO is not well defined.

Figure 23-13

Specimen radiograph of a multilobular osteochondrosarcoma arising from the vertical ramus of the mandible in a dog. These tumors have a granular radiographic appearance often referred to as "popcorn ball."

Metastatic Tumors of Bone

Almost any malignant tumor can metastasize to bone via the hematogenous route. The lumbar vertebrae, femur, humerus, rib, and pelvis are common sites for cancer spread, possibly because these are predilection sites for bone metastasis from the common urinogenital malignancies such as prostate, bladder, urethral, and mammary cancer.[253,254] Metastatic lesions in long bones frequently affect the diaphysis, likely because of the proximity to a nutrient foramen. Nuclear scintigraphy is a sensitive technique to detect bone metastasis. A whole-skeleton bone scan is recommended when metastatic bone cancer is suspected because it is common for multiple sites of metastasis to be present, even if the patient is symptomatic for only one bone.

Benign Tumors of Bone

Osteomas

Osteomas are benign tumors of bone.[343] Radiographically these are well circumscribed, dense bony projections, which are usually not painful to palpation. Histologically, they are composed of tissue nearly indistinguishable from reactive bone. The diagnosis is made after considering the historical and physical exam, as well as radiographic and histological findings. The most important differential diagnosis is MLO when the lesion occurs on the skull. Treatment for osteoma is simple surgical excision, which is usually curative.

Multiple cartilaginous exostosis

Multiple cartilaginous exostosis (MCE) is considered a developmental condition of growing dogs.[17] There is evidence that the etiology of this condition may have a heritable component.[344,345] The actual incidence of MCE is difficult to determine since affected dogs may show no signs and the diagnosis is often incidental. Lesions occur by the process of endochondral ossification when new bone is formed from a cartilage cap analogous to a physis. Lesions are located on bones, which form from endochondral ossification, and lesions stop growing at skeletal maturity. Malignant transformation of MCE lesions has been reported, but generally they remain as unchanged, mature, bony projections from the surface of the bone from which they arose.[322]

Dogs typically present because of a nonpainful or moderately painful palpable mass on the surface of a bone or bones. The pain and lameness is thought to be due to mechanical interference of the mass with the overlying soft tissue structures. In the case of MCE of vertebral bodies, animals can present with clinical signs associated with spinal cord impingement.[346] Radiographically there is a bony mass on the surface of the affected bone, which has a benign appearance with fine trabecular pattern in the body of the mass (Figure 23-14). To obtain a histologic diagnosis, biopsy material must be collected so sections can include the cartilaginous cap and the underlying stalk of bone. Histologically, this cartilaginous cap gives rise to an orderly array of maturing bone according to the sequence of endochondral ossification. The cortical bone surfaces of the mass and the adjacent bone are confluent.[321] A strong presumptive diagnosis is made by evaluation of the physical findings, history, and radiographic findings.

Treatment involves conservative surgical excision, but this is only necessary if signs do not abate after the dog is skeletally mature. Because of the likelihood of a heritable etiology, affected dogs should not be bred. Owners should also be advised of the possibility of late malignant transformation. Dogs with a previous history of MCE should be carefully evaluated for bone malignancy if signs return later in life.

Bone cysts

Cysts are rare, benign lesions of bone. The majority of the veterinary literature pertaining to bone cysts centers on several small series of cases or single case reports.[347-351] Affected animals are often young and present
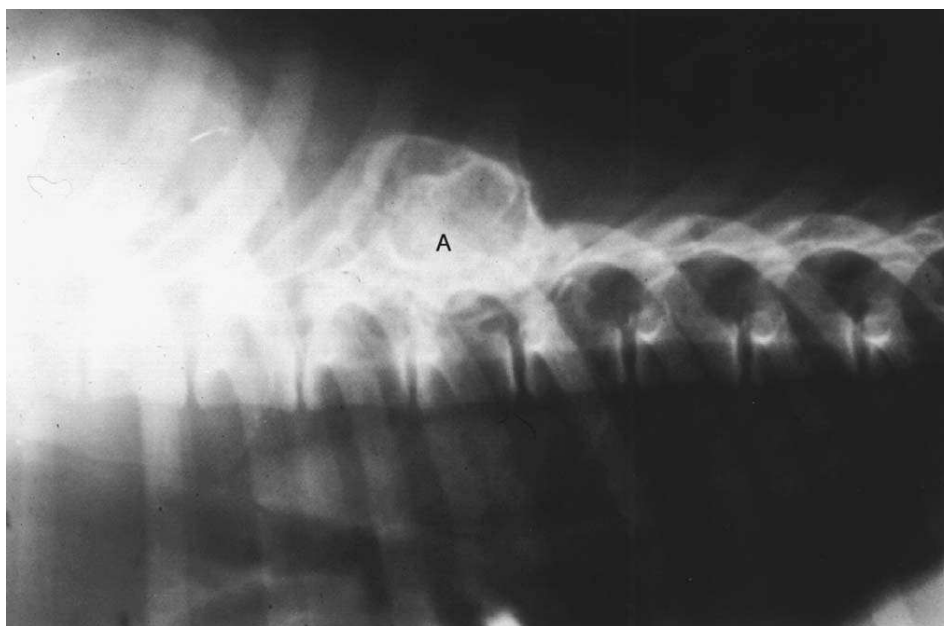
Figure 23-14

Lateral radiograph of a multiple cartilaginous exostosis lesion of the dorsal spinous process in a dog (**A**).

because of mild or moderate lameness; however, pathological fracture can occur through cystic areas of long bones leading to severe lameness. There appears to be a familial tendency in Doberman pinschers and Old English sheepdogs. The nomenclature in various reviews of canine bone cysts is confusing. By definition, a cyst is a fluid-filled sac lined by epithelium. The only true cyst of primary intraosseous origin is a simple bone cyst (SBC, or unicameral bone cyst). These lesions are usually in metaphyseal regions of long bones, and they can adjoin an open growth plate. Sometimes, however, unicameral bone cysts can be diaphyseal or epiphyseal. Neither the etiology nor the pathogenesis is known, but it is speculated that the lesions may be the result of trauma to the growth plate interfering with proper endochondral ossification. Others have theorized that with the rapid resorption and deposition of bone occurring in the metaphysis of a young animal, a cyst might develop if resorption is so rapid that a focus of loose fibrous tissue forms. The focus of fibrous tissue may then obstruct the thin-walled sinusoids, causing interstitial fluid to build up and form a cyst. The theory that appears to be partially substantiated is the synovial "rest" thesis.[352] It is suggested that during fetal development a "rest" of synovial or perisynovial tissue becomes misplaced or incorporated into the adjacent osseous tissue. If this tissue remains or becomes functional, subsequent synovial secretion results in a cyst developing in the bone. Cysts have been described in bone just below articular cartilage (subchondral bone cysts or juxtacortical bone cysts).[350-353] In these, it has often been possible to

demonstrate direct communication with the articular synovial membrane. Radiographically, SBC are single or, more commonly, multilocular, sharply defined, centrally located, radiolucent defects in the medullary canal of long bones. Variable degrees of thinning of the cortex with symmetrical bone "expansion" is often a feature of the radiographs (Figure 23-15). The diagnosis cannot be reliably made from interpretation of radiographs alone. Lytic OS can be misdiagnosed as SBC. Diagnosis of an SBC relies on the histologic finding of a thin, fibrous wall lined by flat to slightly plump layers of mesothelial or endothelial cells. Treatment consists of meticulous curettage and packing the space with autogenous bone graft.

Aneurysmal bone "cysts" (ABCs) are spongy, multiloculated masses filled with free-flowing blood. The walls of an ABC are rarely lined by epithelium, and the lesion is possibly an arteriovenous malformation. A proposed pathogenesis of ABCs is that a primary event such as trauma or a benign bone tumor occurs within the bone or periosteum. This event disrupts the vasculature, resulting in a rapidly enlarging lesion with anomalous blood flow, which damages the bone mesenchyme. The bone reacts by proliferating. As the vascular anomaly becomes stabilized the reactive bone becomes more consolidated and matures.[352] It is important to differentiate these lesions from OS or other malignant lesions of bone. The age of affected dogs ranges from 2 to 14,[354] but it has been reported in a 6-month-old dog.[355] Treatment can be achieved by en bloc resection and reconstruction, but extensive curettage with packing of the defect with autogenous bone graft can be effective.

Figure 23-15

Lateral radiograph of a distal radial bone lesion histologically confirmed as a simple bone cyst. Typical radiographic features include a multilocular, sharply defined, centrally located radiolucency, without evidence of cortical destruction.

PRIMARY BONE TUMORS OF CATS

Incidence and Risk

Cancer involving bones of cats is rare. An estimate of the incidence of all bone tumors in cats is 4.9 per 100,000.[356] Anywhere between 67% to 90% of bone tumors in cats are histologically malignant, and tumors occur in long bones approximately twice as often as in axial skeleton sites.[357] However, in one study, 50 of 90 skeletal OS cases were appendicular with 40 of 90 being axial.[358] There is a report of an extraskeletal OS in the flank of a cat, one case in the liver, and another report of extraskeletal OS in the duodenum.[359-361] Osteosarcomas account for 70% to 80% of all primary malignant cancer of cats. The disease in cats differs from that in dogs in that the primary lesions occur more often in hind limbs in cats and the disease is reported to be far less metastatic than in dogs; however, the authors have seen several cases develop widespread metastatic disease. Osteosarcoma generally affects older cats (mean 8.5[357] and 10.2 years[362]), but the age range of reported cases is large (1 to 20 years). Conflicting reports on gender predisposition exist.[362,363] Osteosarcoma was reported to arise after a limb fracture was repaired with an intramedullary pin in one cat and following radiation therapy in another.[43,364] Multiple cartilaginous exostosis (MCE), is a disease that occurs after skeletal maturity in cats. This is in contrast to dogs where exostoses develop before closure of growth plates. Also, in contrast to dogs, the lesions seldom affect long bones, are rarely symmetric, and are probably of viral rather than familial origin. There does not appear to be any breed or sex predisposition, although early reports of this condition were in the Siamese.[364,365] Affected cats range in age from 1.3 to 8 years (mean 3.2 years).[363] Virtually all cats with multiple cartilaginous exostosis will test positive for FeLV antigenemia. This disease has an aggressive natural behavior.

Pathology and Natural Behavior

Osteosarcoma of cats is composed of mesenchymal cells embedded in malignant osteoid. There may be a considerable amount of cartilage present and osteoid may be scant. A feature of some feline OS cases is the presence of multinucleate giant cells, which may be numerous. Reactive host bone and remnants of host bone are often present in specimens. Tumors are seen to be invasive; however, some surrounding soft tissue may be compressed rather than infiltrated. There is often variation of the histological appearance within the tumor with some portions having more fibrosarcomatous appearance and others more cartilaginous. Some authors have described subtypes that resemble those seen in dogs: chondroblastic, fibroblastic, and telangiectatic as well as the giant cell variant. These features, however, do not appear to confer any prognostic predictive value.[366,367] Osteosarcomas in cats can be of the juxtacortical type. In a large case study, 56 of 146 cases were extraskeletal in origin with the most common site being intrascapular. In this subset, vaccination at the site was considered a predisposing factor. No mention is made of any differences in the pathology of these tumors in comparison to skeletal sites.[358]

In cats with OS of a limb where there are no clinically detectable metastatic lesions, amputation alone may be curative. In one study of 15 cats, the median survival after amputation alone was 24 months.[362] In another study, median survival was 44 months following

amputation.[357] The metastatic potential is much less than for the same disease in dogs or humans. Axial sites carry a poorer prognosis, presumably due to increased difficulty of local resection and control.[358]

Osteochondroma may occur as a solitary lesion in cats, but there is a form that is multicentric (osteochondromatosis). The lesions are composed of hard, irregular exostoses having a fibrous and cartilaginous cap.[363,368] Endochondral ossification occurs from the cartilage cap, which extends to a variable thickness. This cap tends to blend with adjacent tissue making its surgical removal difficult. Cats usually develop multiple sites of disease and there is a potential for malignant transformation and metastasis. The presence of FeLV antigenemia is also foreboding for these cats.

History and Clinical Signs

The most common signs of OS are deformity and lameness depending on the location of the lesion. The lesions may appear radiographically similar to the OS in dogs; however, some cats have lesions arising from the periosteal surface (juxtacortical OS).[357] It is rare for cats to have metastatic osteosarcoma at presentation.

Cats with virally associated multiple cartilaginous exostosis have rather rapidly progressing, conspicuous, hard swellings over affected sites causing pain and loss of function. Common sites for lesion development are the scapula, vertebrae, and mandible; however, any bone can become affected. Radiographically, the lesions are either sessile or pedunculated protuberances from bone surfaces with indistinct borders with the normal bone. There may be a loss of smooth contour with evidence of lysis, particularly if there is malignant transformation.

Diagnostic Workup

Both OS and MCE may be suspected by the radiographic appearance of the lesions and the FeLV status of the cat. Definitive diagnosis is made by histopathologic evaluation of properly collected biopsy tissue. While metastatic rates are lower for cats with primary bone tumors, thoracic radiographs are recommended.

Therapy and Prognosis

For OS of a limb, amputation is recommended. No adjuvant therapy is known to be efficacious, and without adjuvant treatment the median survival of cats with OS ranges from 24 to 44 months.[357,362] The prognosis for axial sites is dependent on resectability.

Cats with MCE have a guarded prognosis. Lesions may be removed surgically for palliation; however, local recurrences are common, or new, painful, debilitating lesions may occur. No reliably effective treatment is known for this condition in cats.

Fibrosarcoma, Chondrosarcoma, and Hemangiosarcoma

Fibrosarcoma is the second most common primary bone tumor of cats.[366] Chondrosarcoma is reported to be next in terms of frequency, and hemangiosarcomas rarely involve bones of cats. Little is known about the biological behavior of these rare lesions; however, metastases have been seen in cats with chondrosarcoma and hemangiosarcoma.[362,364,367]

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Tumors of the Endocrine System

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INTRODUCTION

Endocrine tumors can be classified histologically as nonneoplastic hyperplasias, benign adenomas, or malignant carcinomas. These conditions represent points on a spectrum of endocrine cancer progression. Nonneoplastic hyperplasia is the consequence of aberrant secretion of growth- and function-stimulating hormones. Hyperplasia often is focal or nodular rather than diffuse and is not necessarily reversible when the inciting trophic factor is removed, which illustrates the overlap between nonneoplastic and neoplastic endocrine disease.[1] Adenomas and carcinomas grow autonomously in the absence of trophic stimulation. Adenomas typically are solitary nodules, sharply demarcated from the adjacent normal glandular parenchyma by a thin, fibrous capsule. Carcinomas often are larger than adenomas and demonstrate intraglandular invasion, invasion into and through the capsule of the gland, and/or formation of tumor thrombi within vessels. Distinguishing between benign and malignant lesions can be challenging. In particular, the cellular morphologic features of adenomas and carcinomas often are similar, which makes cytologic samples, small biopsies, and even entire tumors difficult to classify accurately.[1]

PATHOGENESIS OF ENDOCRINE TUMORS

Endocrine tissue is distinct from nonendocrine tissue in several ways that influence the pathogenesis of cancer. Most endocrine tissues are stable rather than renewable. They are not maintained by stem cells, but rather retain the ability to expand when the necessary stimulus is applied and then return to a relatively quiescent state when the trophic substance is removed. Growth (cellular enlargement and division) and function (production of hormone) therefore are tightly linked and controlled by the same physiologic stimulus. This characteristic is manifested by longer intermitotic intervals compared to stem cells, resulting in a lower mutation rate. However, because endocrine tissues are not renewing and have a lower overall tissue turnover rate, any mutations acquired are retained for longer periods.[2] Prolonged stimulation of mutation-bearing endocrine tissue by a trophic substance can result in additional mutations and transformation, resulting in tumor development. Clinically this is seen when longstanding hyperplasia ultimately progresses to neoplasia. In this situation, growth and function within the endocrine organ become dysregulated and ultimately proceed independent of trophic hormone stimulus.

The genetic regulation that dictates expansion and regression of endocrine tissue on demand involves multiple checkpoints. Tumor suppressor genes are believed to limit the expansion of endocrine tissue under normal conditions, and loss of tumor suppressor gene function may lead to neoplasia. Surprisingly, mutations in p53, one of the major tumor suppressor genes, are rare in endocrine tumors; researchers are searching for additional tumor suppressor genes in endocrine tissue.[3] Oncogene activation is another potential mechanism that may disconnect growth from intrinsic control in endocrine tissue and permit a higher rate of second mutations that result in transformation. Mutations and chromosomal translocations that result in oncogene activation, with subsequent signaling protein and transcription factor alterations, have been identified in human endocrine tumors such as thyroid carcinoma, pituitary adenomas, and multiple endocrine neoplasia syndromes.[3]

Growth factors, such as growth hormone (GH), insulin-like growth factors (IGFs), and epidermal growth factor (EGF), also play an integral role in the pathogenesis of endocrine neoplasia in humans.[4] However, normally differentiated and neoplastic endocrine cells respond similarly to growth factor stimulation, and it is unlikely that any single growth factor can cause transformation of a cell. In oncogenesis, therefore, growth factors are thought to play the role of tumor promoter, meaning that growth factor–stimulated hyperplasia increases the probability of mutational events that eventually may release the cell from growth control.

PITUITARY TUMORS

With the exception of corticotroph adenomas in dogs, cancer rarely develops in the adenohypophysis or neurohypophysis in dogs and cats. In these animals, the only primary tumors of the pituitary gland with any clinical impact are tumors of the corticotroph and somatotroph cells. In both species, functional and nonfunctional tumors may develop from these cell types. Treatment usually is sought for animals with nonfunctional tumors when the mass is large enough to induce neurologic signs, such as an altered state of consciousness (obtundation, stupor), behavioral abnormalities (pacing, circling, head pressing, aggression, anorexia), gait abnormalities (ataxia, paraparesis), seizures, and focal cranial nerve abnormalities (blindness, anisocoria, strabismus).[5-9] Local invasion and compression or destruction of adjacent normal pituitary tissue can result in selective hypopituitarism or panhypopituitarism, characterized by hypothyroidism, hypoadrenocorticism, gonadal atrophy, and central diabetes insipidus.[9,10] Animals with functional tumors may have clinical signs related to hormonal hypersecretion in addition to neurologic deficits. In dogs with pituitary macroadenomas, clinical signs associated with the endocrinopathy precede neurologic deficits in 50%; neurologic and endocrine signs are identified simultaneously in 20% to 30%; and neurologic deficits precede a clinical endocrinopathy in 10% to 20%.[11]

Other tumors may secondarily affect the pituitary gland by local extension, including craniopharyngiomas (intracranial germ cell tumors), primary bone tumors, meningiomas, gliomas, ependymomas, and craniopharyngeal duct cysts. Metastatic foci from lymphoma, melanomas, mammary gland carcinomas, and hemangiosarcomas may be seen.[1,12]

HYPERADRENOCORTICISM (CUSHING'S SYNDROME)

Pathology

Hyperadrenocorticism is the most commonly diagnosed endocrinopathy in dogs. It is rare in cats. Of dogs and cats with naturally occurring Cushing's syndrome, 80% to 85% have pituitary-dependent hyperadrenocorticism (PDH).[11,13,14] Excessive secretion of adrenocorticotropic hormone (ACTH) by the pituitary causes subsequent bilateral adrenal hyperplasia and excessive secretion of glucocorticoids. PDH is caused by a functional pituitary tumor. Theoretically, PDH could also result from a primary hypothalamic disorder that excessively stimulates pituitary corticotrophs, but this has not been convincingly documented in dogs or cats. Most functional pituitary tumors in dogs and cats are corticotroph (chromophobe) adenomas that arise from the pars distalis; in dogs, adenomas also occasionally arise from the pars intermedia.[1,11,14] Pituitary carcinomas are rare and usually nonfunctional. Approximately 40% to 50% of dogs with PDH have a microscopic pituitary tumor (microadenoma).[15-17] In the remaining 50% to 60%, a nodule can be visualized using computed tomography (CT), dynamic contrast-enhanced CT, or magnetic resonance imaging (MRI).[7,15-17] Tumors range from 3 mm to several centimeters in diameter. Approximately 10% to 20% of dogs with PDH have a macroadenoma that is 10 mm or larger in diameter.

Of dogs and cats with Cushing's syndrome, 15% to 20% have adrenal-dependent hyperadrenocorticism (ADH).[11,14,18] Excessive secretion of glucocorticoids by a functional adrenocortical adenoma or adenocarcinoma occurs independent of pituitary control, with secondary atrophy of the normal adrenocortical cells in both the affected and contralateral adrenal glands. It is estimated that up to 80% of functional adrenal tumors are adenocarcinomas,[18-23] but distinguishing between benign and malignant lesions often is difficult.[1,11,22,24] Adenomas typically range from 1 to 6 cm in diameter, whereas carcinomas potentially can grow even larger. In one study, adenocarcinomas were more likely to show a trabecular growth pattern, peripheral fibrosis, capsular invasion, necrosis, and/or hemorrhage.[22] They were less likely to show cytoplasmic vacuolization, extramedullary hematopoiesis, or fibrin thrombi. The presence of mineralization was not a distinguishing feature, because it is identified in approximately half of both adenomas and adenocarcinomas.

Approximately 20% of adrenocortical carcinomas locally invade the phrenicoabdominal vein, renal vein, and/or caudal vena cava.[19,21,22,24] Intravascular invasion has the potential to cause life-threatening intra-abdominal or retroperitoneal hemorrhage.[25,26] Metastasis is identified in 50% of dogs with adrenocortical carcinomas.[18-24] Although the liver and lungs most commonly are involved, other organs reported to be affected by metastasis include the kidneys, ovaries, mesenteric lymph nodes, peritoneal cavity, and thyroid gland.

Approximately 1% of dogs diagnosed with hyperadrenocorticism have concurrent functional pituitary and adrenocortical tumors, which complicates the diagnosis of PDH versus ADH.[27] In addition, although uncommon, bilateral adrenocortical adenomas and adenocarcinomas have been reported in dogs.[20,21,24,28] Pheochromocytoma has been diagnosed concurrently in 11 dogs with PDH and 12 dogs with ADH.[20,29,30]

Clinical Features and Diagnostic Evaluation

Several excellent reviews discuss the clinical features, diagnosis, and treatment of hyperadrenocorticism in

dogs and cats.[11,14,31,32] The reader is directed to these reviews for more detailed information.

Hyperadrenocorticism most commonly occurs in middle-aged and older dogs. More than 75% of dogs with PDH and 90% of dogs with ADH are older than 9 years of age. A slight female predilection is seen for both conditions. Approximately 75% of dogs with PDH weigh less than 20 kg, whereas up to 50% of dogs with ADH weigh more than 20 kg. However, considerable overlap exists. Poodles, dachshunds, terriers, and German shepherds are among the most commonly affected breeds for each condition. Similar to dogs, cats with hyperadrenocorticism are middle aged or older. The reported age range for cats with PDH is 5 to 16 years, and that for ADH is 8-15 years.[14,32] No breed or gender predilection is seen in cats.

Clinical signs usually progress slowly over months to years. The most commonly identified clinical signs include polyuria/polydipsia, polyphagia, lethargy, pendulous abdominal profile, and hair coat changes (alopecia, slow regrowth of shaved hair, pyoderma, and calcinosis cutis in dogs; alopecia secondary to grooming, slow regrowth of shaved hair, pyoderma, and skin fragility in cats). Cats often have a history of diabetes mellitus that is difficult to regulate[33-35]; this likely represents a combination of insulin antagonism and similarities in the clinical signs associated with diabetes mellitus and hyperadrenocorticism. Concurrent neurologic abnormalities may also be present in patients with pituitary macroadenomas (see preceding discussion of pituitary tumors).

Common changes identified in dogs by a baseline complete blood count (CBC), chemistry panel, and urinalysis include stress leukogram, thrombocytosis, elevated liver enzymes (alkaline phosphatase, alanine transferase, aspartate transferase), hypercholesterolemia, hyperglycemia, and isosthenuria. Abnormalities are less consistently identified in cats, and liver enzyme elevations are uncommon. Thoracic radiographs are usually unremarkable but may reveal bronchial mineralization or pulmonary metastasis associated with adrenocortical carcinoma.[36] Abdominal ultrasonography should not be used as a routine screening test for hyperadrenocorticism, but in a patient otherwise diagnosed with Cushing's syndrome, it can help differentiate PDH from ADH.[37-40] In patients with PDH, the adrenal glands are relatively equal in size with normal or enlarged dimensions. Their normal shapes are retained, and they are homogeneous and hypoechoic compared with the renal cortex. With adrenal tumors the affected gland is enlarged and rounded or irregularly rounded. Intravascular invasion also may be identified. The atrophied contralateral gland may be thin and narrow or not visualized at all. Complete evaluation of the abdomen may also reveal changes consistent with steroid hepatopathy, intra-abdominal metastasis, or both.

Several endocrinologic tests have been evaluated for their usefulness as a diagnostic tool for hyperadrenocorticism. Among the most commonly used screening tests are the urine cortisol:creatinine ratio, ACTH stimulation test, and low-dose dexamethasone suppression test (LDDS) (Table 24-1).[31,41-43] All these tests are relatively sensitive, but false negative results occasionally occur. Nonadrenal illness can significantly affect the pituitary-adrenal axis, causing false positive results.[44,45] The ACTH stimulation test is accepted as the most specific test, followed by the LDDS and then the urine cortisol:creatinine ratio.

A diagnosis of Cushing's syndrome and the decision to pursue treatment should be based on a combination of the clinical history, physical examination findings, baseline blood work and urinalysis, and endocrine test results. Once a diagnosis of hyperadrenocorticism has been confirmed, endogenous plasma ACTH levels, the high-dose dexamethasone suppression test (HDDS), and abdominal ultrasonography can be used to determine whether the cause is PDH or ADH (see Table 24-1).[18,37-40,43]

Treatment

Management of hyperadrenocorticism must be put in context with any other medical problems the dog or cat may have. Few dogs with Cushing's syndrome require urgent correction of cortisol levels, and in some situations immediate treatment of the condition may not be in the patient's best interest. Management of other disorders may take priority. However, once more urgent conditions have been addressed, ultimate control of hyperadrenocorticism must be achieved to prevent the catabolic and immunosuppressive effects of hypercortisolemia from further debilitating the patient or compromising successful treatment of concurrent disease.

Surgery

Hypophysectomy is the treatment of choice for PDH in humans, and it has been advocated for PDH in dogs.[11,46] Transsphenoidal hypophysectomy was described in 84 dogs with PDH.[47,48] Perioperative mortality occurred in six dogs, and treatment failure as a result of incomplete resection occurred in another six. In the 72 dogs in which hyperadrenocorticism went into remission, 91% remained disease free at 1 year and 80% at 2 years. Hypophysectomy also has been reported in seven cats with PDH.[48,49] Two cats died perioperatively, and two with persistent oronasal fistulas died within 8 months of surgery. Clinical signs recurred in one cat 19 months after surgery. Two cats were alive and disease free at 15 and 46 months after surgery. Significant complications reported included prolonged or permanent diabetes insipidus (hypernatremia) and

TABLE 24–1   Expected Endocrine Testing Results in Dogs and Cats with Hyperadrenocorticism

	Pituitary-Dependent Hyperadrenocorticism (PDH)	Adrenal-Dependent Hyperadrenocorticism (ADH)	Comments
Screening tests to diagnose hyperadrenocorticism			
Urine cortisol: creatinine ratio	Increased	Increased	Sensitive but not specific
Low-dose dexamethasone suppression test (LDDS)	No suppression or transient suppression of plasma cortisol level	No suppression of plasma cortisol level	
Adrenocorticotropic hormone (ACTH) stimulation test	Elevated poststimulation plasma cortisol level	Elevated poststimulation plasma cortisol level	More specific but less sensitive than LDDS
Tests to differentiate PDH from ADH			
Endogenous ACTH concentration	Increased or high normal	Decreased or low normal	
High-dose dexamethasone suppression test (HDDS)	Suppression of plasma cortisol level	No suppression of plasma cortisol level	Of dogs with PDH, 25% do not show suppression.
Abdominal ultrasonography	Bilateral adrenal enlargement	Unilateral adrenal mass	Should not be used as a screening test

hypothyroidism in dogs, and persistent oronasal fistula formation in cats.[47-49] The authors of these studies acknowledged a steep learning curve with this technique, and access to centers with expertise in this procedure currently is very limited.

Adrenalectomy is the treatment of choice for dogs with ADH.[19-21,24] The ideal surgical candidate is not compromised by sequelae from hypercortisolemia and has a noninvasive, nonmetastatic adrenocortical adenoma. Patients severely compromised by Cushing's syndrome may be treated presurgically with ketoconazole or o,p′-DDD (see the following section on chemotherapy), but this is rarely necessary. The perioperative mortality rate initially was reported to be 50%,[19] but more recent studies report a rate of 20%.[20,21,24] This likely reflects improvement in the prophylactic management of life-threatening complications such as hypocortisolemia, pancreatitis, hemorrhage, and pulmonary thromboembolism. Dexamethasone should be administered intraoperatively and continued postoperatively. An ACTH stimulation test should be performed the day after surgery to assess the function of the pituitary-adrenal axis; the test result should be low after a successful surgery. Glucocorticoid supplementation should be continued until ACTH stimulation results return to within the low normal range (usually 2 to 5 months). Mineralocorticoid supplementation is needed less frequently and for shorter periods, but

postoperative monitoring of electrolytes is still indicated. Intraoperative and postoperative administration of heparin and plasma, followed by hetastarch, has been advocated to reduce the frequency of pulmonary thromboembolism.[24]

The long-term prognosis is good for patients that survive the perioperative period, have a low postoperative ACTH stimulation result, and have complete clinical resolution of the Cushing's syndrome. Of these patients, 80% to 90% have no clinical recurrence of hyperadrenocorticism. Intravascular invasion has not been shown to negatively affect the prognosis.[24] In addition, some patients with confirmed metastatic disease at the time of adrenalectomy still have complete resolution of clinical signs for several months after surgery.[21,24] If metastatic disease is confirmed or if clinical signs persist or recur, chemotherapy treatment is recommended (see the following section).

Bilateral adrenalectomy is not recommended for dogs with PDH because other treatment options are equally or more effective and have fewer risks. However, bilateral adrenalectomy is the most successful treatment for cats with PDH.[35] Symptoms improve and insulin requirements decrease, although lifelong supplementation with glucocorticoids and mineralocorticoids is required. Management of clinical signs of hypercortisolemia with metapyrone before surgery may reduce the risks of anesthesia and improve postoperative recovery.[50]

Chemotherapy

Conventional therapy for dogs with PDH and nonsurgical or metastatic ADH involves administration of o,p'-DDD (mitotane [Lysodren]).[8,23,51,52] o,p'-DDD is a potent adrenocorticolytic drug that causes severe necrosis of the zona fasciculata and zona reticularis, with relative sparing of the mineralocorticoid-producing zona glomerulosa. The drug's bioavailability is poor, and administration with meals is recommended. Once absorbed, o,p'-DDD undergoes extensive hepatic metabolism. Concurrent administration of other drugs that alter hepatic microsomal enzymes may affect the efficacy of treatment (e.g., phenobarbital may inhibit the action of o,p'-DDD).

It is strongly recommended that readers unfamiliar with o,p'-DDD therapy read an excellent, detailed review before administering the drug to patients.[11] The standard protocol begins with a loading dose of 50 mg/kg/day, PO divided and given twice daily. An ACTH stimulation test is performed after 8 to 10 days or sooner if the patient shows any decrease in appetite or water consumption, vomiting, diarrhea, or listlessness. Ideally, the post-ACTH cortisol level should be 1 to 5 μg/dl. Once this goal has been achieved, maintenance therapy begins, at a dosage of 25 to 50 mg/kg/week, divided as most convenient. Monitoring with ACTH stimulation tests should be performed 1 and 3 months after the start of maintenance therapy and then every 3 to 4 months or when clinical signs recur.

o,p'-DDD is a very effective treatment for canine PDH. Approximately 95% of dogs respond within 5 to 9 days of starting the loading dose, and almost all within 14 days. Recurrence of clinical signs of hyperadrenocorticism is reported in 50% of dogs within the first year of starting maintenance therapy.[51] When this occurs, increasing the maintenance dosage of o,p'-DDD by 25% usually is sufficient; occasionally a reinduction loading dose also is needed. The mean survival time with o,p'-DDD therapy is approximately 2.5 years, and dogs frequently live several years longer and die of unrelated causes.[11,51] Approximately 25% to 35% of dogs died or were euthanized because of sequelae of hyperadrenocorticism, such as congestive heart failure, diabetic complications, infection, and pulmonary thromboembolism. Another 20% died of neurologic complications associated with a pituitary macroadenoma.[11,51]

Treatment with o,p'-DDD is not as effective for ADH.[23,52] Only 20% of dogs respond as desired within 10 days of starting the loading phase. The remainder require longer induction periods or higher induction doses, or both. The maintenance dosage selected for a given patient depends on the response during loading. Most patients experience relapse of clinical signs, requiring reinduction followed by an increased maintenance dosage. At high dosages the drug itself can induce anorexia, vomiting, diarrhea, and rarely central neurologic signs. Overall median survival is about 1 year. Patients with visible metastasis have a more guarded prognosis, although regression of metastatic disease has been reported with o,p'-DDD therapy.[23]

Other treatment options for dogs with hyperadrenocorticism are available if o,p'-DDD is not effective or if the dog is unable to tolerate the drug. Trilostane and ketoconazole, inhibitors of steroid biosynthesis, are both good alternatives.[53-56] L-Deprenyl (selegiline [Anipryl]), a monoamine oxidase inhibitor that increases dopamine concentrations, has minimal efficacy and is not recommended.[57,58]

o,p'-DDD is not an effective treatment for hyperadrenocorticism in cats, and there is a risk of chlorinated hydrocarbon toxicity in this species.[32] Trilostane, metapyrone, and ketoconazole are reasonable alternatives, although experience with these drugs is limited.[14,50,59-61]

Radiation therapy

Up to 10% to 20% of dogs with PDH have macroadenomas, and the severity of neurologic signs correlates with the size of the tumor relative to the size of the cranial vault.[9] Irradiation of the pituitary gland results in significant reductions in neurologic signs. In a study in which 24 dogs with pituitary macroadenomas received a total of 48 Gy, 10 dogs experienced complete resolution of neurologic signs, and 10 achieved partial remission.[9] The overall median progression-free survival time was 13 months; however, patients with only mild or moderate neurologic deficits had a median progression-free survival time of 21 months. Patients in which the relative tumor size was 12% or less of the cranial vault volume were more likely to achieve complete remission and were four times less likely to experience tumor progression. Endocrine-active tumors were six times less likely to progress. Radiation therapy inconsistently reduces tumor secretion of ACTH, and many patients still require medical management of the Cushing's syndrome.[9,62,63]

Of dogs that have PDH without accompanying neurologic signs, 50% to 60% have a visible pituitary mass that is identifiable on CT or MRI scans. In two studies, 13 dogs with PDH were imaged with MRI at initial diagnosis and then again 1 year later.[15,64] Five dogs did not have a visible tumor on initial imaging; 1 year later, two of these dogs had visible tumors measuring 6 and 7 mm in diameter. Of the eight dogs with visible tumors on initial imaging, four had tumors that were significantly larger 1 year later. Clinical signs at presentation, the results of endocrinologic testing, and the response to medical management did not predict the tumor size or rate of growth. Because a small relative tumor size strongly predicts the efficacy of radiation therapy, pituitary imaging has been advocated for all patients diagnosed with PDH.

PITUITARY SOMATOTROPH TUMORS (FELINE ACROMEGALY)

Acromegaly, or chronic overgrowth of connective tissue, bone, and viscera caused by chronic excessive secretion of growth hormone (GH), is uncommon in dogs and cats. Many effects of GH are mediated by insulin-like growth factor-I (IGF-I, also called *somatomedin C*).[65] The liver is a major site of IGF-I synthesis, and much of the circulating IGF-I is derived from the liver. IGF-I also is produced in most other tissues, where it has important paracrine actions. GH and IGF-I work synergistically to stimulate cell proliferation in the skeleton and soft tissues, and IGF-I promotes protein synthesis. GH also has direct catabolic effects on carbohydrates and lipids. GH antagonizes insulin via a postreceptor effect, resulting in impaired glucose uptake and decreased carbohydrate utilization.

In cats, the most common etiology of acromegaly is a functional pituitary adenoma arising from somatotroph cells.[65,66] In contrast, canine acromegaly most commonly results from progestin-induced GH secretion by the mammary ductular epithelium.[67] Only one published case report has described a GH-producing pituitary adenoma in a dog;[68] therefore canine acromegaly is not discussed further.

Feline acromegaly most often occurs in older male domestic shorthair or domestic longhair cats, although the reported age ranges from 4 to 17 years.[65,66] Clinical signs reflect the catabolic and diabetogenic effects of GH and the anabolic effects of IGF-I. The earliest and most common clinical signs are polyuria, polydipsia, and polyphagia resulting from the concurrent diabetes mellitus. Weight loss may occur initially, but eventually weight gain usually is seen. Extreme insulin resistance is common, with doses exceeding 2 units/kg having little effect on blood glucose levels. Eventually the anabolic effects of IGF-I become evident, resulting in enlargement of the head, prognathia inferior, diffuse thickening of the oropharyngeal soft tissues, hepatomegaly, renomegaly, adrenomegaly, hypertrophic cardiomyopathy, clubbing of the paws and distal extremities, and degenerative arthropathy. Neurologic signs may also be identified, caused by a macroadenoma compressing adjacent structures in the brain (see the earlier discussion of pituitary tumors).

Acromegaly should be suspected in any cat with insulin-resistant diabetes mellitus, particularly if the cat has been gaining weight or has any conformational changes indicative of GH and IGF-I excess. Definitive diagnosis requires documentation of an increased serum GH concentration, but unfortunately, no commercially available GH assay exists for cats. A serum IGF-I assay is readily available (Diagnostic Center for Population and Animal Health Endocrine Diagnostic Section, Michigan State University, Lansing, Michigan) and is a useful screening test in cats.[33,65] CT or MRI also may be valuable; 17 of 18 reported cats that underwent imaging had visible pituitary masses, which ranged from 4 to 12 mm in diameter.[33,66,69,70] A diagnosis of PDH must be ruled out before a tentative diagnosis of acromegaly can be made (see earlier discussion of hyperadrenocorticism).

A consistently effective treatment plan for feline acromegaly has not been identified. Radiation therapy has been reported in five cats.[66,69] Two cats had no response to treatment, but three experienced complete resolution of the diabetes mellitus and normalization of the serum GH concentration. One cat showed recurrence of clinical signs 6 months after radiation therapy; the other two were still in remission 16 and 28 months after treatment. Transsphenoidal hypophysectomy has been described for cats with corticotroph adenomas, but it has not been reported for cats with somatotroph tumors.[48,49] One report of successful transsphenoidal cryotherapy exists.[70] Octreotide has not been effective in cats.[66] Bromocriptine, a dopamine agonist used in humans with acromegaly, has not been evaluated in cats.

The long-term prognosis for cats with acromegaly is guarded. From the time the diagnosis of acromegaly is established, the median survival time is approximately 20 months.[66] Among the most common causes of death are congestive heart failure secondary to hypertrophic cardiomyopathy, renal failure secondary to glomerular changes caused by poorly regulated diabetes mellitus or GH excess, and neurologic deficits induced by a pituitary macroadenoma.

ADRENAL GLAND NEOPLASIA

Primary adrenal tumors were reported in approximately 0.17% to 0.76% of pet dogs (representing 1% to 2% of all canine tumors) and 0.03% of cats (representing 0.2% of feline tumors) from the Veterinary Medical Database.[71] Metastasis to the adrenal glands was reported uncommonly in that database. In contrast, a retrospective study that included all dogs and cats that underwent complete necropsy evaluation during a 20-year period at the University of California–Davis reported that 195 (41%) of 472 neoplastic canine adrenal lesions were adrenocortical tumors, 151 (32%) were pheochromocytomas, and 126 (27%) were metastatic lesions.[72] Of 20 feline adrenal neoplastic lesions, 6 (30%) were adrenocortical tumors, 2 (10%) were pheochromocytomas, and 12 (60%) were metastatic lesions. Fewer than half of these metastatic lesions were grossly visible at necropsy. Lymphoma was the most common cancer to spread to the adrenal glands in both species. Other metastatic tumors commonly identified in the dog included hemangiosarcoma, mammary carcinoma, histiocytic sarcoma, pulmonary carcinoma, and melanoma. The right and left adrenal glands were

affected equally, as were the cortex and the medulla. The only notable exception was that all metastatic melanomas were restricted to the adrenal medulla.

Improved imaging technology and increased experience with abdominal ultrasonography have greatly enhanced the ability to identify both clinical and subclinical adrenal abnormalities.[24,39,73] Based on this experience, it appears that the adrenal gland is affected with neoplasia more commonly than was previously appreciated. Masses that exceed 2 cm in diameter, that diffusely distort the shape of the entire affected gland, and that invade adjacent vasculature are more likely to be malignant. However, the absence of these features does not rule out malignancy. Approximately half of all adrenocortical adenomas and adenocarcinomas are mineralized; pheochromocytomas rarely mineralize.

CT and MRI are the imaging modalities of choice for humans with adrenal tumors.[74,75] Both modalities are sensitive, consistently identifying lesions as small as 1 cm in diameter. Invasion of adjacent organs and vasculature also can be readily visualized. MRI provides the additional advantage of distinguishing adrenocortical tumors from adrenal medullary tumors; the latter often appear much brighter on T_2-weighted images. Experience with these imaging modalities in dogs with adrenal lesions is limited.[75-78] However, their continued use likely will provide additional information about the characteristics of specific adrenal lesions to aid in diagnosis and treatment planning (Figure 24-1).

Advances in abdominal imaging have led to the diagnostic dilemma of the incidental adrenal mass. When an incidental adrenal mass is identified, a thorough history and physical examination are indicated, including blood pressure measurement and fundic examination. Endocrinologic testing may be pursued if a functional adrenocortical tumor is suspected (see the earlier discussion of hyperadrenocorticism). Given the high incidence of metastatic lesions, imaging of the thorax and abdomen should be performed to rule out another primary tumor. Aspiration cytology and ultrasound- or CT-guided biopsies are not routinely recommended for small adrenal lesions because of the high risk of complications and the inability to differentiate reliably between benign and malignant lesions.[39,74] In humans, surgical removal of the affected adrenal gland is indicated if the lesion is suspected to be a functional adrenal tumor, if the lesion exceeds 4 cm in diameter, or if any evidence of vascular invasion exists.[74] If none of these criteria are met, imaging is repeated in 3 to 6 months. A similar algorithm should be adopted for veterinary patients. When dogs with incidental adrenal masses eventually underwent necropsy, 30% had no abnormalities that were detected grossly or histologically, 40% had nonfunctional benign tumors or granulomas, and 30% had pheochromocytomas.[11]

ADRENOCORTICAL TUMORS

Most adrenocortical tumors in dogs and cats are functional. Increased glucocorticoid levels are responsible for the constellation of clinical signs and biochemical abnormalities associated with Cushing's syndrome. In addition to secreting cortisol, most functional adrenocortical tumors concurrently secrete one or more sex hormones, including androstenedione, progesterone, 17-hydroxyprogesterone, and testosterone, but not estradiol.[79] On occasion, dogs and cats may have functional adrenocortical tumors that secrete sex hormones without glucocorticoids.[80-82] These patients still have clinical signs typical of Cushing's syndrome, but the results of the ACTH stimulation and LDDS tests are normal. Serum sex hormone assays are commercially available (University of Tennessee Clinical Endocrinology Service, Knoxville, Tennessee). Adrenalectomy is the treatment of choice.

Aldosterone-secreting adrenocortical tumors, resulting in Conn's syndrome, are rare in both dogs and cats.[11,83-85] The most common presenting sign is episodic weakness, and serum chemistry reveals hypokalemia and a normal to elevated sodium concentration. Patients also are frequently hypertensive. Medical management with the aldosterone antagonist spironolactone has had variable success. Adrenalectomy is the treatment of choice, with some patients experiencing long-term control.

Adrenocortical Tumors in Ferrets

Adrenocortical tumors are the second most common tumor in ferrets, with a reported prevalence of 25%.[86,87] More than two thirds are benign nodular hyperplasias or adenomas, and the remainder are malignant adenocarcinomas.[87-89] These tumors typically secrete sex hormones, including estradiol, 17-hydroxyprogesterone, androstenedione, and/or dehydroepiandrosterone.[89-93] Cortisol and corticosterone levels are normal both before and after ACTH stimulation.[89-92] There is a female predilection, and in both genders neutered animals are affected more commonly than intact animals.[86-89] Neutering releases negative feedback on the pituitary gland, and the resulting increased secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) can potentially promote tumor formation in sensitive adrenal gland cells.[89,93] Artificially long photoperiods associated with indoor housing may contribute as well; light cycles longer than 8 hours have been shown to stimulate release of gonadotropin-releasing hormone (GnRH) and LH in ferrets.[94]

Patients typically present around 4 years of age.[87-89] The most common clinical signs are bilaterally symmetric alopecia with or without pruritus and in females, vulvar enlargement with or without mucoid discharge. Other frequently observed signs include lethargy and

muscle atrophy. In males, return of intact behaviors may be seen, and less commonly stranguria may occur secondary to prostatic enlargement or cyst formation. Clinical signs initially may be seasonal, appearing in the spring and resolving in the fall, but eventually they become persistent.[90]

Baseline CBC and chemistry panels usually are unremarkable, although anemia and thrombocytopenia occasionally develop secondary to elevated estradiol levels.[87,89] Abdominal radiographs are not helpful, and an adrenal mass is successfully visualized on abdominal ultrasonography in only half of affected patients.[89,95,96] Measurement of serum estradiol, 17-hydroxyprogesterone, and androstenedione concentrations is commercially available (University of Tennessee Clinical Endocrinology Service). In one study, 98% of ferrets with bilaterally symmetric alopecia, vulvar enlargement, or return to intact male behavior were confirmed to have adrenocortical neoplasia.[87] It therefore is reasonable to go forward with abdominal exploratory surgery based on the clinical signs alone.

Surgery is the treatment of choice for hyperadrenocorticism in ferrets.[87-89,95] In patients with unilateral disease, the left adrenal gland is affected more than twice as frequently as the right. Approximately 10% to 20% of patients have bilateral involvement at the time of initial surgery. When bilateral disease is identified, the larger gland is removed completely, and a subtotal adrenalectomy (50% to 60%) is performed on the contralateral gland. Complete bilateral adrenalectomy has a fair to poor success rate in ferrets.[87] All adrenal tissue removed should be submitted for histopathology, because up to half of patients with bilateral tumors have different histologies in each adrenal gland.[87,88] Distant metastasis is rare, but adrenocortical carcinomas may locally invade the liver, kidney, and/or vena cava. Ligation and segmental resection of the vena cava has been performed without complication.[87,88]

The perioperative mortality rate is less than 10%.[87-89,95] Most patients recover uneventfully, and fewer than 5% require postoperative glucocorticoid or mineralocorticoid supplementation. Clinical signs typically resolve within 1-2 months. In one study, 13 of 79 ferrets (17%) that had unilateral disease had recurrence of clinical signs 3 to 14 months after surgery as a result of the development of a functional tumor in the contralateral adrenal gland.[87] Partial adrenalectomy of the remaining gland is recommended. In another study, 55 ferrets with bilateral disease (regardless of whether they initially presented with bilateral disease) all experienced complete resolution of clinical signs after subtotal bilateral adrenalectomy.[88] Clinical signs subsequently recurred in 8 of the ferrets (15%) 7 to 22 months after surgery.

Medical management is recommended for ferrets that have nonresectable tumors or that experience recurrence of clinical signs postoperatively.

o,p'-DDD and ketoconazole, drugs commonly used to treat hyperadrenocorticism in dogs, are ineffective in ferrets.[87-89] Leuprolide acetate (Lupron Depot) is a GnRH analog that inhibits the production and release of FSH and LH by downregulating GnRH receptors in the pituitary gland. In one study, a dose of 100 μg given intramuscularly (100 to 150 μg/kg) significantly reduced serum sex hormone levels and resulted in marked improvement or complete resolution of clinical signs.[93] The duration of efficacy varied, with clinical signs recurring anywhere from 1.5 to 8 months after a single treatment. Leuprolide acetate does not have any direct cytotoxic effects and would not be expected to cause a reduction in tumor size.

Ferrets with adrenocortical neoplasia often are diagnosed with concurrent diseases. Up to 25% of ferrets with adrenocortical neoplasia are diagnosed with an insulinoma either at initial presentation for the adrenocortical tumor or within the following several months (see the discussion of pancreatic beta-cell tumors later in the chapter).[87-89,93] This underscores the importance of thoroughly evaluating all abdominal organs if an adrenalectomy is performed. Cardiomyopathy is also common, affecting up to 10% of ferrets with adrenocortical disease.[87,89,95] The reason these diseases appear to be related is unknown.

ADRENAL MEDULLARY TUMORS

Pheochromocytomas are tumors that arise from chromaffin cells and secrete catecholamines, predominantly norepinephrine or a mixture of norepinephrine and epinephrine, based on data from humans.[97,98] These tumors are uncommon in dogs, accounting for 0.01% to 0.1% of all canine tumors, and rare in cats.[99-101] Pheochromocytomas typically originate from the adrenal medulla, but they may also arise from extraadrenal neural crest cells. Extraadrenal pheochromocytomas, also called *paragangliomas*, have been reported infrequently in both dogs and cats[30,102,103] and account for up to 10% of all chromaffin tumors in humans.[74] In humans, the most common extraadrenal site is the organ of Zuckerkandl, located adjacent to the inferior (caudal) mesenteric artery. The heart, carotid bodies, tissues adjacent to the aorta, and urinary bladder may also be affected.

More than half of canine pheochromocytomas are considered malignant.[24,30,99] Thirty percent to 50% invade adjacent vasculature (Figure 24-2); metastasis is less common, occurring in 20% to 30% of dogs. The liver, spleen, and lungs are affected most often, and other reported sites include the regional lymph nodes, kidneys, bone, pancreas, peritoneum, brain, spinal cord, and heart.[24,30,99,104,105] Up to 5% of affected dogs have bilateral adrenal gland involvement.[30,99] Concurrent adrenal

medullary and adrenocortical tumors also have been reported.[20,29,30] Immunohistochemical staining for chromogranin A and synaptophysin can be used to distinguish pheochromocytomas from adrenocortical tumors; pheochromocytomas stain positive with high sensitivity and specificity.[24,30,106]

Pheochromocytomas usually are diagnosed in older dogs (median age, 11 years).[24,30,99] The diagnosis is made as an incidental finding at necropsy in as many as 50% to 60% of affected patients.[30,99] Forty percent to 50% of dogs have clinical signs associated with increased secretion of catecholamines, but unfortunately, the most commonly identified clinical signs and physical examination abnormalities are nonspecific: weight loss, anorexia, panting, tachypnea, tachycardia, lethargy, and collapse.[30,98,99] In addition, these signs often are paroxysmal, reflecting intermittent catecholamine release by the tumor. Up to 10% to 15% of patients have clinical signs referable to the presence of a space-occupying mass, including abdominal distension, abdominal pain, ascites, peripheral limb edema and, rarely, severe, acute intra-abdominal or retroperitoneal hemorrhage.[25,30,99]

Hypertension is demonstrated in up to half of all dogs with pheochromocytomas.[30,98,99] It often is paroxysmal; therefore repeated blood pressure measurements and fundic examinations increase the likelihood of detection. A CBC, chemistry panel, and urinalysis do not reveal consistent abnormalities. Thoracic radiographs usually are within normal limits but occasionally reveal cardiomegaly secondary to concentric hypertrophy or distension of the caudal vena cava as a result of tumor thrombus. Pulmonary metastasis is identified in 5% to 10% of patients.[30,99] Abdominal ultrasound scans are very helpful, because many patients are diagnosed with pheochromocytoma only after the discovery of an incidental adrenal mass. Ultrasonography also identifies intravascular invasion with high sensitivity and specificity.[24] CT and MRI are the imaging modalities of choice for humans with pheochromocytomas, and early experience with these techniques in canine patients has been encouraging (Figure 24-1).[75]

Plasma and 24-hour urine concentrations of catecholamines and their metabolites (metanephrine, normetanephrine, and vanillylmandelic acid) routinely are measured in humans, and elevated concentrations strongly support the diagnosis of pheochromocytoma.[107] These tests are not readily available for dogs or cats, and results were ambiguous in one reported dog.[99] Radioactive iodine-labeled metaiodobenzylguanidine (^{131}I-MIBG) scintigraphy also is commonly used in humans.[74] MIBG is structurally similar to norepinephrine and is readily taken up by cells of neural crest origin. A pheochromocytoma has been successfully imaged in one dog using [123]iodine-labeled MIBG scintigraphy and in two dogs using positron emission tomography (PET) with a fluorinated analog of MIBG.[108,109]

Surgery is the only definitive treatment for pheochromocytoma.[24,30,98,99,110] Hypertension, tachycardia, and arrhythmias are commonly seen during induction of anesthesia and surgical manipulation of the tumor. Dogs should receive phenoxybenzamine, a noncompetitive alpha-adrenergic antagonist, for 1 to 2 weeks before surgery.[24,97] The standard dosage of phenoxybenzamine is 0.5 mg/kg PO given every 12 hours, but a dosage as high as 2.5 mg/kg every 12 hours may be needed to control hypertension. If the dog is tachycardic, a beta blocker, such as propranolol (0.2 to 1 mg/kg every 8 hours) or atenolol (0.2 to 1 mg/kg PO every 12 to 24 hours), also may be administered. These drugs should be started after alpha-adrenergic blockade has been initiated to prevent unopposed alpha-adrenergic stimulation and severe hypertension. In two studies in which dogs were not pretreated, the overall perioperative mortality was about 40%.[30,110] By comparison, when dogs were pretreated, perioperative mortality decreased to less than 20%.[24] Dogs without metastatic disease that survive the perioperative period often enjoy long-term survival, although in rare cases metastatic disease can develop several years after surgery.[30]

For patients with extensive tumor burdens, surgical debulking, including removal of tumor thrombi from the vena cava, may reduce catecholamine levels enough to control clinical signs. If not, alpha- and beta-adrenergic antagonists may be used in addition. In humans, chemotherapy protocols, including streptozotocin and combination cyclophosphamide, dacarbazine, and vincristine, have had variable success.[74] Radiation therapy using high-dose ^{131}I-MIBG also has had only moderate success.[74,108] Chemotherapy and radiation therapy have not been evaluated in dogs with pheochromocytoma.

Up to half of dogs with pheochromocytomas develop concurrent neoplasms.[30,99] Some of these tumors arise from other endocrine tissues, including the pituitary gland, adrenal cortex, thyroid gland, parathyroid gland, and pancreatic beta cells. Multiple endocrine neoplasia (MEN) syndromes have been well characterized in humans (see discussion of MEN later in the chapter), and similar molecular mechanisms may exist in dogs. Interestingly, though, most concurrent tumors identified are derived from nonendocrine tissues. Among the most commonly identified cancers are soft tissue sarcomas, chemodectomas, hepatobiliary tumors, mammary gland tumors, and osteosarcoma.

THYROID GLAND NEOPLASIA IN DOGS

Thyroid tumors account for 1.2% to 3.8% of all tumors in dogs.[111,112] In necropsy studies, an estimated 30% to 50% of canine thyroid tumors are benign adenomas.[111,113]
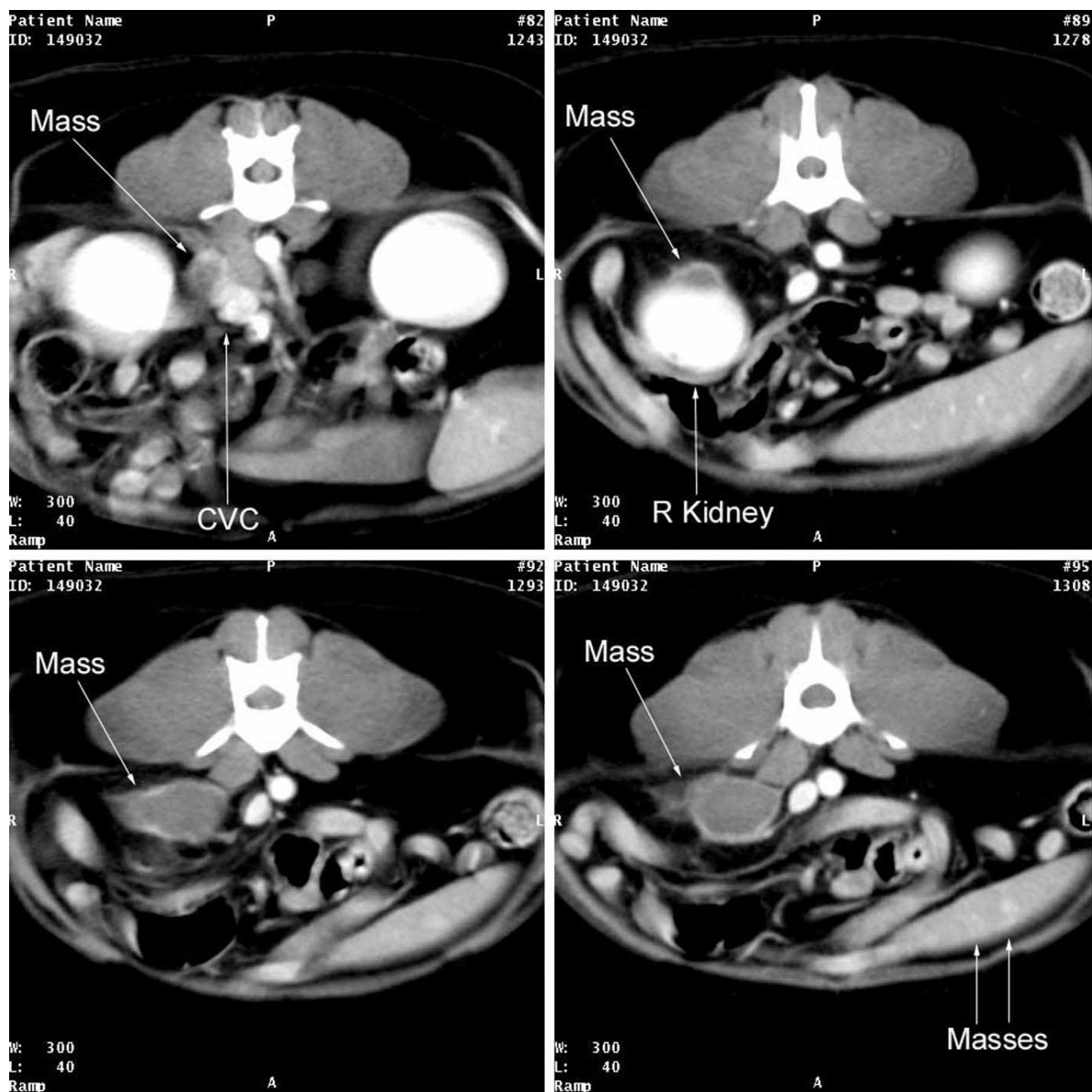
Figure 24-1

Four transverse, contrast-enhanced computed tomography (CT) images of the abdomen from a dog with a right-sided adrenocortical carcinoma (*mass*). The tumor can be seen invading into the caudal vena cava (*CVC*), causing significant retroperitoneal hemorrhage surrounding the right kidney and extending caudally. An incidental renal cyst associated with the right kidney also can be identified (*upper right*).
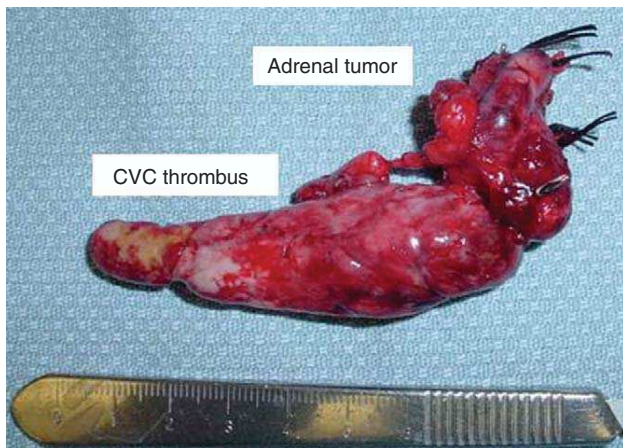
Figure 24-2

Left-sided pheochromocytoma with a 7-cm caudal vena caval (*CVC*) thrombus that was surgically removed from a Lhasa Apso. Despite the locally invasive behavior of this tumor, the dog remains disease-free more than 3 years after surgery.

Most adenomas are small, noninvasive, and clinically silent. Consequently, clinical studies suggest that almost all clinically detectable canine thyroid tumors are histologically malignant.[112,114,115] Thyroid tumors of follicular cell origin are subclassified as papillary, follicular, compact (solid), or anaplastic. All subgroups stain positive for thyroglobulin and thyroid transcription factor-1.[116-118] Papillary carcinomas are most common in humans,[119,120] whereas follicular and compact forms are most common in dogs.[1,112,116,121] Medullary thyroid carcinomas, also called *parafollicular* or *C-cell carcinomas*, are relatively uncommon in both humans and dogs, although one study reported that 36% of canine thyroid tumors were medullary in origin.[118] Positive immunohistochemical staining for calcitonin is the most accurate means of identifying these tumors, but they also often stain positive for calcitonin gene–related peptide, thyroid transcription factor-1, chromogranin A, and neuron-specific enolase.[106,116-118,122]

The etiology of thyroid neoplasia in dogs is largely unknown. Thyroid-stimulating hormone (TSH) may play a contributing role. Canine thyroid tumors retain TSH receptors, and hypothyroid beagles that did not receive thyroid hormone supplementation had an increased incidence of thyroid tumors.[123,124] Thyroid irradiation is associated with an increased incidence of thyroid tumors in all species, including humans, rodents, and dogs.[1,114,119,120] The molecular pathogenesis of thyroid neoplasia is best defined in humans.[119,120] Activation of receptor tyrosine kinases (e.g., RET and TRK) is common in papillary carcinomas, activating mutations in RAS are frequently identified in follicular carcinomas, and inactivation of p53 is common in anaplastic carcinomas. Sporadic and germline RET mutations are also associated with sporadic and MEN-2–associated medullary thyroid carcinoma, respectively. In dogs, one report identified a p53 mutation in one of 23 primary thyroid carcinomas.[125] Another report confirmed trisomy 18 in a canine thyroid adenoma.[126]

Thyroid tumors typically develop in older dogs (median reported age, 9 to 11 years).[112,121,127-129] A gender predilection has not been reported, but boxers, golden retrievers, and beagles are at increased risk.[112] The right and left lobes are affected with equal frequency, and as many as 60% of patients have bilateral involvement.[127,130] In rare cases, ectopic thyroid tissue can produce tumors in the tongue, ventral neck, cranial mediastinum, and heart base.[130-133] Up to 35% to 40% of dogs have visible metastatic disease at initial presentation, and as many as 80% ultimately develop metastasis.[112,118,130,134,135] The lungs and regional lymph nodes (including the retropharyngeal, cranial cervical, and mandibular lymph nodes) most often are affected, but a wide variety of tissues can be affected. Medullary carcinomas may have a lower metastatic potential than follicular and solid carcinomas.[118]

Most canine thyroid carcinomas are nonfunctional. Based on clinical signs and serum thyroxine (T_4) concentrations, approximately 60% of patients are euthyroid, 30% are hypothyroid secondary to destruction of the normal thyroid parenchyma, and 10% are hyperthyroid.[114,129,136,137] Higher percentages of functional (hyperthyroid) tumors are reported in Australia.[137b] Most dogs are presented for a palpable ventral cervical mass (Figure 24-3).[112,118,121] Less common abnormalities include coughing, rapid breathing, dyspnea, dysphagia, dysphonia (change in bark), and facial edema (i.e., precaval syndrome [Figure 24-4]). Acute severe hemorrhage can occur secondary to invasion of the cervical vasculature.[138] In addition to clinical signs referable to the physical thyroid mass, dogs with hyperthyroidism frequently exhibit polyphagia, weight loss, muscle wasting, polyuria, and polydipsia.[114,136,137]

The differential diagnosis for a mass in the region of the thyroid gland in dogs includes abscesses or granulomas, salivary mucoceles, lymphatic metastasis from tonsillar squamous cell carcinoma, lymphoma, carotid body tumor, and sarcomas. In humans, thyroid cytology is very accurate for identifying thyroid tumors and distinguishing between benign and malignant types.[119,120] However, the accuracy of cytology in dogs with thyroid masses is not good. Cytology confirms the mass to be of thyroid origin in only half of affected dogs, and definitive recognition of malignancy often is not possible.[112,114] Malignant thyroid tumors have a higher vascular density than normal thyroid tissue and benign tumors,[129] and hemodilution is a common

Figure 24-3

Freely movable, noninvasive thyroid carcinoma in a dog. Surgical removal alone resulted in local and systemic control for longer than 4 years.

Figure 24-4

Precaval syndrome, resulting in pronounced facial edema, secondary to an invasive thyroid carcinoma in a boxer.

problem. That being said, aspiration methods that reduce the likelihood of hemodilution ("needle without the syringe") and careful inspection of the feather edge result in a diagnosis of "endocrine" neoplasia in most cases. The increased vascularity also adds significant risk to large-core needle biopsy procedures.

Routine staging for dogs with thyroid carcinoma includes a general health assessment with laboratory evaluation (CBC, chemistry panel, and urinalysis), three-view thoracic radiographs, and cytologic or histologic evaluation of the regional lymph nodes. Cervical ultrasonography can be used to confirm that a mass is of thyroid origin and to assess invasiveness and vascularity.[139,140] The retropharyngeal and cranial cervical lymph nodes also can be examined for evidence of metastasis. Scintigraphy with technetium-99m (^{99m}Tc)-pertechnetate or, less commonly, radioactive iodine (^{131}I) is used primarily to identify local residual disease after surgery, ectopic tumors, or metastatic disease.[112,127,130,131] Most primary tumors are visualized, although the pattern of uptake often is heterogeneous. Metastatic disease is identified less consistently. To be visualized with scintigraphy, a thyroid tumor must be capable of trapping ^{99m}Tc-pertechnetate or of trapping and organifying ^{131}I. It may or may not be able to complete the remaining steps necessary for synthesis and secretion of functional thyroid hormone.

Treatment of canine thyroid carcinomas is dictated by the size of the mass, the extent of invasion (mobility; compare Figure 24-3 and Figure 24-5), the presence or absence of gross metastatic disease, and any concurrent symptoms of thyrotoxicosis (Figure 24-6). Surgical excision provides the best outcome with the least morbidity when tumors are freely moveable without deep tissue invasion.[118,121,141] Thyroidectomy is not recommended when the tumor invades adjacent structures, including vasculature, recurrent laryngeal nerves, vagosympathetic trunk, parathyroid glands, and occasionally the larynx and trachea (Figure 24-7). Bilateral, fixed disease is particularly difficult to resect without significant morbidity, including damage to recurrent laryngeal nerves that results in laryngeal paralysis and aspiration pneumonia. Extensive hemorrhage can result from the

Figure 24-5

Large, fixed, invasive thyroid carcinoma in a dog (head is toward the top).

vascularity of the tumor, invasion into adjacent blood vessels, and local coagulopathies.[129,141] It is estimated that only 25% to 50% of thyroid carcinomas are surgically amenable at the time of initial diagnosis.[118,121] Median survival after thyroidectomy is about 3 years if the tumor is freely moveable, 6 to 12 months if the tumor is more invasive.[118,121]

Nonresectable thyroid carcinomas can be managed with radiation therapy. External beam radiation therapy is used most often. One study evaluated definitive radiation therapy (48 Gy, delivered in 4 Gy fractions on an alternate day schedule) in 25 dogs with unresectable thyroid carcinomas and no visible metastasis.[127] Tumors either stabilized or decreased in size. The time to maximal tumor reduction ranged from 8 to 22 months in dogs with tumors that responded to treatment. The progression-free survival rates were 80% at 1 year and 72% at 3 years. The first cause of failure was local progression in three dogs, metastasis in four dogs, and concurrent local progression and metastasis in three dogs. Limited information exists regarding the use of definitive radiation in the adjuvant or neoadjuvant settings.[128,142] The radiation therapy protocol just described was evaluated in an additional eight dogs, seven of which had undergone incomplete thyroidectomy
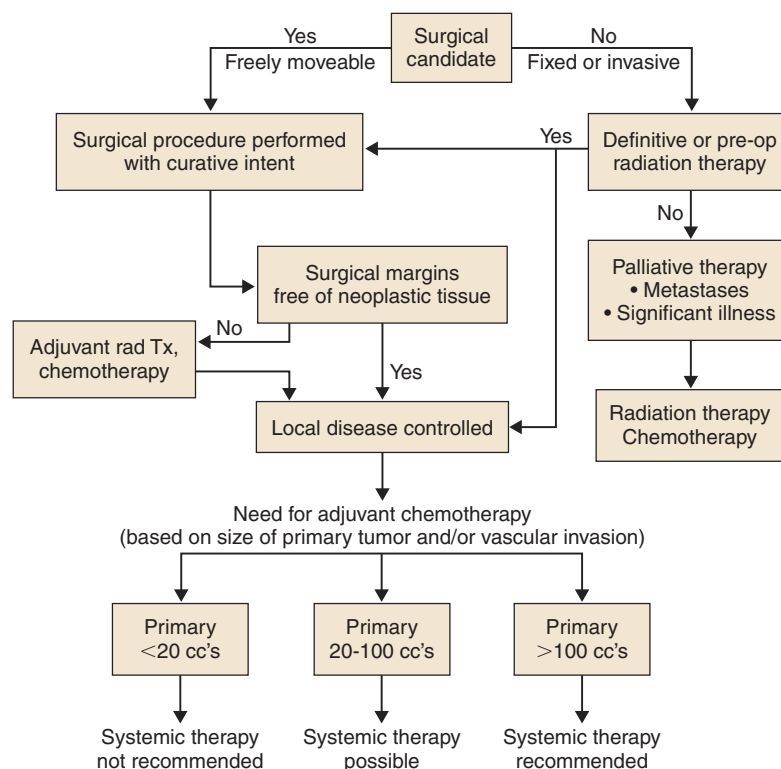
Figure 24-6

Treatment for thyroid carcinoma (clinical and histologic staging completed).
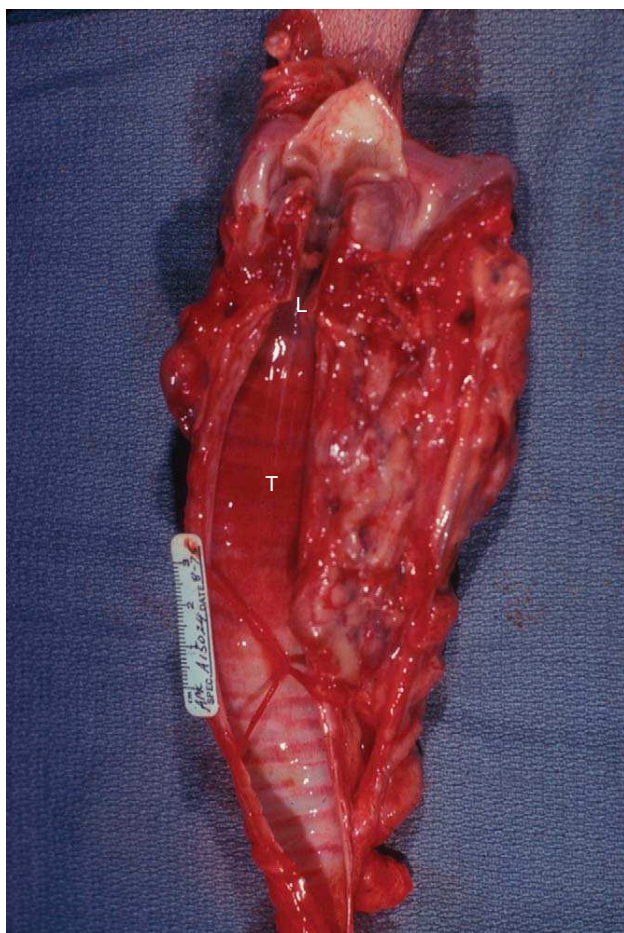
Figure 24-7

Invasion of a thyroid carcinoma into the larynx *(L)*, trachea *(T)*, and cervical blood vessels (postmortem specimen).

before irradiation.[128] The median survival time was just over 2 years (range, 1 to 3 years). None of the dogs developed local recurrence, although four died of metastatic disease. Radiation-induced toxicoses to the larynx, trachea, and esophagus usually are well tolerated. Hypothyroidism may develop months to years after treatment.[127,142]

For dogs with gross metastatic disease, hypofractionated radiation therapy may provide effective palliation of the primary tumor. In a study evaluating palliative radiation therapy as the sole treatment modality, 13 dogs received 36 Gy in four weekly 9 Gy fractions.[134] Complete reduction of the primary tumor occurred in one dog, and partial reduction occurred in nine dogs. Local progression occurred in five dogs 11 to 24 months after irradiation. Gross metastatic disease was present in five dogs at initial presentation and developed in two more dogs during the study. The overall median survival time was about 22 months,

and local and metastatic progression occurred equally in all dogs.

In humans with well-differentiated thyroid carcinoma, ^{131}I routinely is administered postoperatively to destroy occult microscopic local or metastatic carcinoma.[119,120] Experience with ^{131}I thyroid ablation in dogs is very limited but appears to be effective when tumors are capable of iodide trapping and organification.[137b,141,143,144] The doses of ^{131}I used for thyroid ablation in dogs (usually around 100 mCi) are significantly higher than those used to treat cats with hyperthyroidism (see the discussion on thyroid gland neoplasia in cats later in the chapter); consequently few facilities are able to offer ^{131}I treatment for dogs.

Chemotherapy has been evaluated in dogs with thyroid tumors. Of dogs treated with either doxorubicin or cisplatin, 30% to 50% demonstrated a partial response (i.e., a greater than 50% reduction in volume).[135,145,146] Individual responses have also been reported with the use of mitoxantrone or actinomycin D.[147,148] Chemotherapy may be considered for dogs with large, nonresectable primary tumors or gross metastatic disease or both. Metastatic potential increases significantly when the primary tumor volume exceeds 27 cm^3 and approaches 100% when tumor volume exceeds 100 cm^3.[113] Bilateral tumors are 16 times more likely to metastasize than unilateral tumors.[127] For patients at increased risk of developing metastasis, systemic chemotherapy should be considered even if local control is achieved with surgery and/or definitive radiation therapy.

THYROID GLAND NEOPLASIA IN CATS

Hyperthyroidism (thyrotoxicosis) is the most common endocrine disorder in cats.[149,150] It is almost always caused by a primary thyroid abnormality that results in the production and secretion of excessive T_4 and triiodothyronine (T_3). Multinodular adenomatous hyperplasia is identified histologically in 70% to 75% of thyrotoxic cats[1,149-153] Both thyroid lobes usually are affected, although they may be asymmetrically enlarged at the time of diagnosis. Solitary benign adenomas are the underlying cause in 20% to 25% of hyperthyroid cats. Malignant carcinomas are the least common cause of hyperthyroidism, occurring in only 1% to 3% of thyrotoxic cats[1,149,153-155] Nonfunctional thyroid carcinomas also are uncommon.[154,155] As with dogs, follicular and compact carcinomas are identified most often. Feline thyroid carcinomas are more locally invasive than their benign counterparts, and their metastatic rate is up to 70%, with regional lymph nodes and lungs most often affected.[154,156]

TSH stimulates growth and differentiation, including thyroid hormone synthesis and secretion, via heterotrimeric G-protein and cyclic adenosine

monophosphate (cAMP) signal transduction pathways.[157] Decreased expression of the inhibitory subunit $G_{i\alpha}$ has been identified in hyperplastic feline thyroid glands, implying that cAMP levels might be inappropriately increased.[158] Mutations in the TSH receptor itself have not been documented.[159] Overexpression of one or more RAS oncogenes was identified in adenomas and hyperplasias from 18 of 18 cats diagnosed with hyperthyroidism but not in adjacent normal thyroid tissue or in thyroid tissue from 14 unaffected cats.[160] In the same tumors that overexpressed RAS, Bcl-2 and p53 proteins were undetectable using immunohistochemistry.

Hyperthyroidism was not recognized as a clinical disorder in cats until 1979, yet currently it is estimated to affect as many as 1 in 300 cats.[149] This may reflect a true increase in incidence, heightened awareness and testing by veterinarians, or both. If a true increased incidence has occurred, environmental factors may have contributed. Several have been associated with hyperthyroidism, including the consumption of commercially prepared, canned cat food, indoor residence, use of cat litter, and exposure to herbicides, fertilizers, and topical flea products; however, none has been conclusively incriminated as a primary inciting cause.[161-163]

A brief overview of the clinical features, diagnosis, and treatment of feline hyperthyroidism is presented here, with an emphasis on malignant thyroid tumors. Several excellent and detailed reviews discuss feline hyperthyroidism, and the reader is directed to these for additional information.[149,150]

Most hyperthyroid cats are older; 95% are 8 years old or older at initial diagnosis.[151] There is no gender predilection, but Siamese and Himalayan breeds are at decreased risk.[162] The most common signs noticed by owners include weight loss, polyphagia, polydipsia, polyuria, gastrointestinal signs (vomiting, diarrhea, increased stool production), and increased activity.[149-151,164] Additional abnormalities commonly identified on physical examination include the presence of a palpable thyroid nodule, cardiovascular abnormalities (tachycardia, heart murmur, gallop rhythm, premature beats), and poor hair coat. A definitive diagnosis routinely is based on an elevated serum total T_4 level.[151,164,165] Fewer than 10% of hyperthyroid cats have a total T_4 level within the reference range. Total T_4 levels that do fall within the reference range usually are due to normal fluctuations in serum thyroid hormone concentrations and/or the presence of concurrent nonthyroidal illness, which may lower the measurable total T_4.[165-167] If hyperthyroidism is suspected in a cat with a normal total T_4 level, the total T_4 should be measured again in 1 to 2 weeks in addition to evaluation of free (non-protein-bound) T_4 (fT_4). If a diagnosis is still not confirmed and nonthyroidal illness has been ruled out, a triiodothyronine (T_3) suppression test or thyrotropin-releasing hormone (TRH) stimulation test

should be considered.[168-170] Thyroid function tests cannot be used to distinguish between benign and malignant tumors.

Staging for cats with hyperthyroidism should include a baseline CBC, chemistry panel, and urinalysis. Up to 20% to 40% of hyperthyroid cats are azotemic, and in these cats, resolution of thyrotoxicosis may cause clinical deterioration of renal function.[151,164] Additional diagnostics routinely recommended include thoracic radiography, electrocardiography, echocardiography, and blood pressure measurement.[171,172] ^{99m}Tc-pertechnetate scintigraphy is very useful for determining the anatomic extent of functional thyroid tissue and for planning therapy.[152] Unilateral uptake occurs in cats with a solitary adenoma and atrophy of the normal contralateral gland. Bilateral uptake, even if asymmetric, is indicative of adenomatous hyperplasia. Ectopic thyroid tissue also can be identified. The presence of more than two masses in the cervical region, uptake in the lungs, or both strongly support a diagnosis of metastatic carcinoma.[154-156]

Several treatment options exist for feline hyperthyroidism. Oral antithyroid medications (e.g., methimazole and carbimazole) inhibit thyroid hormone synthesis, providing rapid, reversible resolution of clinical signs.[173,174] These drugs are ideal for assessing the effect of resolution of hyperthyroidism on renal function and for preparing a cat for anesthesia and thyroidectomy or the prolonged hospitalization required for ^{131}I therapy. They also can be used as a long-term treatment modality. Methimazole is administered at a starting dose of 2.5 mg given orally twice daily for 2 weeks. Based on clinical signs and serum T_4 levels, the dosage can be increased as needed every 2 weeks in increments of 2.5 mg/day. Methimazole compounded in pluronic lecithin organogel (PLO) can be applied topically to the pinna. Absorption is inconsistent, but nonetheless these preparations are clinically effective for some cats.[141,175-177] Approximately 10% to 15% of patients treated with methimazole develop adverse effects, including lethargy, anorexia, vomiting, and blood dyscrasias.[150,173,177] It is important to recognize that oral antithyroid medications block thyroid hormone synthesis without having any cytotoxic effect on thyroid follicular cells; clinical thyrotoxicosis returns within 24 to 72 hours after therapy is discontinued.[173,174] They therefore have limited benefit for cats with thyroid carcinomas, providing only short-term palliation of clinical signs.[154,155]

Definitive therapy currently consists of thyroidectomy or ^{131}I thyroid ablation. Surgical excision of the affected thyroid lobe or lobes is a highly effective treatment. Seventy percent to 75% of cats have bilateral disease, but involvement usually is asymmetric. Thyroid scintigraphy is beneficial for determining whether unilateral or bilateral thyroidectomy is necessary. Intracapsular and extracapsular thyroidectomy techniques

have been described.[178,179] Intracapsular techniques better preserve adjacent parathyroid tissue, whereas extracapsular ones more consistently remove all hyperplastic or neoplastic thyroid tissue. Hyperthyroid cats often are poor candidates for anesthesia, and preoperative treatment with oral antithyroid medications may be necessary. Postoperative complications include Horner's syndrome, laryngeal paralysis, hypoparathyroidism (hypocalcemia), and hypothyroidism. All surgically excised tissue should be submitted for histopathology to rule out the presence of a thyroid carcinoma. Cats with thyroid carcinoma that undergo thyroidectomy usually experience improvement in clinical signs, but most remain hyperthyroid or develop recurrent hyperthyroidism within a few months of surgery.[154,155] Adjuvant therapy, such as radioiodine, is recommended for these patients.

[131]I often is regarded as the treatment of choice for cats with hyperthyroidism, particularly those with bilateral thyroid hyperplasia, ectopic thyroid tissue, or thyroid carcinoma.[153-156,180] [131]I has a half-life of 8 days and emits both beta and gamma radiation.[150] Beta particles, which account for 80% of the tissue damage, travel a maximum of 2 mm in tissue and have an average path length of 400 μm. They therefore cause local destruction while sparing adjacent hypoplastic thyroid tissue, parathyroid glands, and other cervical structures. The dose of ^{131}I can be calculated from tracer kinetic studies, but empiric doses are used routinely.[149,153,180] For cats with benign thyroid disease, ^{131}I doses typically range from 2 to 6 mCi based on clinical signs, the serum T_4 concentration, and the size of the thyroid tumor.[153] Using this dosing strategy, fewer than 5% of cats remain hyperthyroid or experience relapse of clinical signs. When this occurs, a second treatment usually is curative. Persistent hypothyroidism requiring thyroid hormone supplementation occurs in approximately 2% of treated cats. Cats with thyroid carcinomas usually have larger tumor burdens, and malignant cells trap and retain iodine less efficiently.[150,155] These cats therefore are treated with higher ablative doses of [131]I (20 to 30 mCi).[149,150,154,155] [131]I thyroid ablation may be more effective when combined with thyroidectomy.[155] For cats with nonfunctional thyroid tumors, external beam radiation therapy may be used alone or in combination with thyroidectomy to provide local control.[154]

Ultrasound-guided percutaneous ethanol injection has also been evaluated. Cats with solitary adenomas have a good response, with resolution of clinical signs persisting for longer than 12 months.[181] Experience with bilateral hyperplasia has been less promising.[182] Injections must be staged to prevent bilateral laryngeal paralysis, and improvements in clinical signs and serum T_4 levels are transient. Similar results have been obtained using ultrasound-guided percutaneous radiofrequency heat ablation.[183]

PARATHYROID NEOPLASIA

Parathyroid neoplasia is uncommon in dogs and rare in cats.[184-188] Tumors usually arise from the parathyroid hormone (PTH)–producing chief cells, resulting in hyperparathyroidism. Rare oxyphil cell tumors have been reported in humans but not in dogs or cats.[189] Parathyroid tumors can be classified histologically as hyperplasias, adenomas, or carcinomas. Clear morphologic characteristics to distinguish between these pathologies do not exist, which supports a model of sequential transformation.[1,184] Approximately 90% of dogs and cats have a solitary parathyroid mass.[1,184-187] These nodules are most commonly classified as adenomatous or hyperplastic, but up to 5% are considered malignant. Metastasis has not been reported. Up to 10% of dogs and cats have two parathyroid nodules.[184,187,190] These masses may or may not be classified similarly on histopathology. Primary hyperplasia involving all four parathyroid glands is rare but has been reported in both species.[188,190]

Primary hyperparathyroidism occurs most commonly in older dogs and cats. Keeshonds are significantly overrepresented; other commonly affected breeds include Labrador retrievers and German shepherds.[185,186] Siamese cats may be at increased risk.[187] Clinical symptoms of primary hyperparathyroidism usually develop slowly as a result of a progressive increase in serum calcium. In dogs, polyuria and polydipsia are the most commonly reported clinical signs, followed by lethargy, weakness, inappetence, and lower urinary tract signs secondary to calcium-oxalate urolithiasis and urinary tract infections.[184-186] The clinical signs in hypercalcemic cats are similar but occur with different frequencies. Anorexia and lethargy occur in up to 70% of affected cats, whereas the incidence of gastrointestinal signs (vomiting, diarrhea, and/or constipation), polyuria and polydipsia, and lower urinary tract signs is about 25% for each of these.[188] Physical examination findings are usually unremarkable in dogs, but a significant number of cats have a palpable parathyroid mass.[187,191-193]

Serum total and ionized calcium values may be mildly to severely elevated in dogs and cats with hyperparathyroidism.[185-187] Serum phosphorus concentrations are inconsistently low or low normal; normal and even mildly elevated concentrations also have been documented.[185-187] Mild to moderate liver enzyme elevations may be present. In uncomplicated hyperparathyroidism, renal function is not compromised, although the urine is isosthenuric or hyposthenuric. Prolonged hypercalcemia can lead to nephrocalcinosis and significant irreversible renal damage.

A diagnosis of primary hyperparathyroidism is made by documenting an inappropriately elevated serum PTH concentration relative to the serum calcium

concentration.[187,194,195] In normal dogs and cats, the serum PTH concentration decreases as the serum calcium concentration increases. Therefore hypercalcemic patients with serum PTH concentrations within the reference range are still considered to have excessive levels of PTH, supporting a diagnosis of primary hyperparathyroidism. Cervical ultrasound also can be used to support the diagnosis. Parathyroid nodules as small as 0.5 cm in diameter are routinely visualized.[140,191,195,196] If ultrasonography suggests a mass, the surgeon almost always can identify the mass; however, a negative ultrasound does not rule out a tumor. Enumeration and localization of the affected gland or glands is beneficial for planning therapy. Double-phase parathyroid scintigraphy with [99mTc]-sestamibi also has been used to localize hyperfunctional parathyroid tissue, but low sensitivity and specificity limit its utility.[197,198]

Dogs and cats with primary hyperparathyroidism may require acute medical therapy for their hypercalcemia before definitive therapy is pursued. The decision to implement symptomatic therapy should be based on the degree of hypercalcemia and the presence of any concurrent calcium-induced sequelae, such as renal insufficiency, cardiac arrhythmia, or neurologic dysfunction. Fortunately, dogs and cats with primary hyperparathyroidism rarely require emergency treatment. (The management of hypercalcemia is discussed in detail in Chapter 5.)

Definitive therapy for primary hyperparathyroidism most commonly involves surgical removal of the affected gland or glands.[185-187] Preoperative cervical ultrasound is recommended to help minimize the time needed to explore the neck at surgery. All the parathyroid glands must be evaluated, because up to 10% of dogs and cats have multiple masses. Three of the four parathyroid glands can be removed without the risk of permanent hypoparathyroidism. For the rare dog or cat with hyperplastic lesions involving all four parathyroid glands, the decision whether to remove all parathyroid tissue should be based on the patient's clinical status and renal function, along with the owner's ability to provide lifelong calcium and vitamin D supplementation.

Ultrasound-guided intralesional ethanol injection and radiofrequency heat ablation are alternatives to surgery. In one study, eight dogs were treated with intralesional ethanol injections.[199] The serum PTH concentration decreased in all eight dogs within 24 hours. The serum calcium level normalized in six patients within 24 hours. The other two dogs had normal calcium concentrations within 5 days, although one required a second intralesional ethanol injection 3 days after the first. In another study, 11 dogs were treated with radiofrequency heat ablation.[200] The serum PTH and calcium levels normalized after a single treatment in six dogs. Two treatments were required for two dogs, and treatment was unsuccessful in three dogs.

Surgical removal or percutaneous ablation of a functional parathyroid tumor causes the serum PTH and calcium concentrations to decline over the following 1 to 7 days.[184,186,199,200] Posttreatment hypocalcemia correlates with the severity of the preoperative hypercalcemia (and hence the severity of the negative feedback exerted on the normal parathyroid tissue). Dogs with a preoperative serum calcium concentration below 14 mg/dl are considered at low risk for clinical tetany. The serum calcium level should still be monitored twice daily, and treatment with vitamin D (dihydrotachysterol) with or without calcium is initiated if the serum calcium level drops below normal or the patient begins to show signs of tetany. Dogs with a preoperative serum calcium concentration above 14 mg/dl may benefit from initiation of prophylactic vitamin D and calcium treatment the day of surgery or percutaneous ablation. The supplements then are gradually tapered over several weeks. Cats may be less susceptible to hypocalcemic tetany, but close posttreatment monitoring of serum calcium levels is still recommended.[187]

The long-term prognosis for dogs and cats with hyperparathyroidism uncomplicated by renal failure is excellent. Fewer than 10% of dogs and cats experience recurrence of clinical signs, and if this does occur, a second surgery or ablative procedure usually is curative.[184] Patients with compromised renal function must be observed closely and managed appropriately. Renal function may or may not improve when the hypercalcemia is corrected.

PANCREATIC BETA-CELL TUMORS (INSULINOMAS)

Functional beta-cell tumors are common in ferrets, uncommon in dogs, and rare in cats.[86,201] The clinical signs associated with these tumors arise from hypersecretion of insulin, but immunohistochemical analysis reveals that these tumors often produce multiple additional hormones, including glucagon, somatostatin, pancreatic polypeptide, and gastrin.[202-204] The biologic behavior of beta-cell tumors often is difficult to predict using routine histologic criteria for malignancy.[1,204] Although they usually have benign histologic features, approximately 50% of canine insulinomas metastasize.[205-208] Regional lymph nodes and the liver are affected most often. Pulmonary metastasis is rare. Up to 15% of affected dogs have multiple pancreatic nodules. In ferrets, insulinomas are rarely metastatic, but multiple pancreatic nodules are identified in up to 75% of affected animals at initial exploratory surgery.[209-211] Beta-cell tumors have been diagnosed in too few cats to allow their biologic behavior to be characterized accurately in this species, but metastasis to regional lymph nodes and the liver has been

reported.[201,212-215] In humans, up to 90% of insulinomas are solitary benign adenomas that are cured by surgical excision.[216]

Beta-Cell Tumors in Dogs

In dogs, insulinomas occur most often in medium and large breeds, including Labrador retrievers, golden retrievers, German shepherds, and Irish setters.[205-207] The median age at diagnosis is about 10 years (reported range, 3 to 14 years), and no gender predilection exists. Clinical signs caused by the associated hypoglycemia include seizures, weakness, collapse, ataxia, muscle fasciculations, and bizarre behavior. Signs are episodic and often exacerbated by fasting, exercise, excitement, and even eating. A paraneoplastic peripheral neuropathy characterized by variable demyelination and remyelination, axonal degeneration, and formation of myelin globules has been described.[217-219] The peripheral neuropathy may be subclinical, or dogs may exhibit flaccid paresis or paralysis, hyporeflexia, and muscle atrophy. Appendicular and facial nerves may be affected.

Confirmation of an insulin-secreting tumor is based on documentation of hypoglycemia (blood glucose level under 60 mg/dl), along with an inappropriately elevated serum insulin concentration (usually exceeding 10 μU/ml). Although most dogs with insulinomas are persistently hypoglycemic, fasting occasionally is needed to ensure that the blood glucose level is below the reference range. When fasted, patients must be closely monitored to prevent a hypoglycemia-induced crisis. Collecting multiple samples for paired glucose and insulin measurements may improve diagnostic accuracy.[220] Use of the insulin:glucose ratio or amended insulin:glucose ratio is not recommended because they lack specificity.[201,205,220]

Abdominal ultrasonography may provide additional evidence to support the diagnosis of insulinoma, but a pancreatic mass is clearly identified in fewer than half of affected dogs[207,221,222] Abdominal ultrasound also has very low accuracy for detecting metastatic lesions; true metastases often are not visualized, and unrelated benign changes often are incorrectly reported as metastases. In one study, CT accurately identified 10 of 14 pancreatic masses in dogs with insulinomas, but its usefulness is limited by a large number of false positive results in screening for metastasis.[221] Scintigraphy and single photon emission computed tomography (SPECT) using radiolabeled somatostatin analogs such as octreotide and pentetreotide have yielded variable results.[221,223-225]

Regardless of imaging results, exploratory laparotomy is indicated in dogs when insulinoma is suspected based on clinical signs, hypoglycemia, and an inappropriately elevated serum insulin concentration.[205-207,226] The goals of surgery are to confirm the diagnosis of insulinoma, determine the stage of disease, and remove as much neoplastic tissue as possible. Before surgery, medical management should be initiated to prevent episodes of severe hypoglycemia. A regimen of feeding frequent small meals and administering glucocorticoids usually is adequate. If the blood glucose level is critically low, intravenous fluids containing 2.5% to 5% dextrose should be administered at a rate of one and one half to two times maintenance. A continuous infusion of glucagon also may be used.[227] (Management of hypoglycemia is discussed in detail in Chapter 5.)

The details of the surgical technique have been described elsewhere.[228] Tumors are identified in both lobes of the pancreas with equal frequency, and up to 15% of dogs have multiple nodules.[206,207] Most canine insulinomas are visible or palpable at surgery (Figure 24-8). If needed, intraoperative ultrasound can help localize small tumors.[221,229] Routine biopsy of the regional lymph nodes and liver should always be performed, and any suspected metastatic lesions should be removed whenever possible. In one study, biopsy samples were obtained from 14 dogs suspected of having metastatic disease based on gross observations at surgery, but metastasis was confirmed histologically in only eight of the dogs.[207]

Medical management for dogs that do not undergo surgery or that have recurrent hypoglycemia after surgery consists primarily of dietary management and glucocorticoid therapy. Prednisone is started at a dosage of 0.25 mg/kg PO given every 12 hours; the dosage can be increased gradually, as needed, to control clinical signs. Diazoxide also has been used with variable success, although it can be prohibitively expensive and difficult to obtain.[201,205,207] Diazoxide inhibits insulin secretion, stimulates hepatic gluconeogenesis and glycogenolysis, and inhibits tissue use of glucose. It does not inhibit
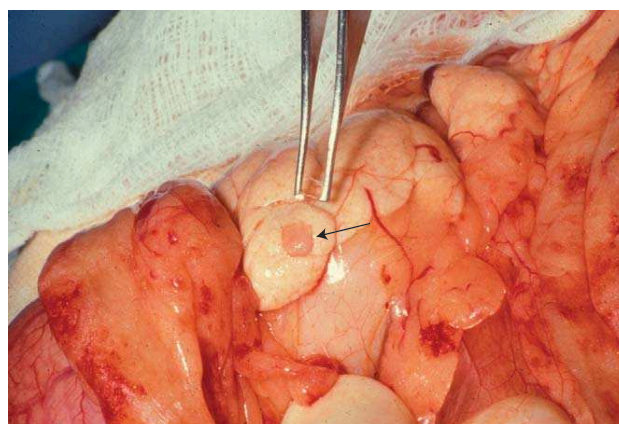
Figure 24-8

Nodular, insulin-secreting pancreatic mass *(arrow)* in a dog.

insulin synthesis and does not have cytotoxic (antineoplastic) effects. A starting dosage of 10 mg/kg PO divided and given twice daily is used; the dosage can be increased to a maximum of 60 mg/kg/day. Octreotide binds to somatostatin receptors and inhibits insulin synthesis and secretion.[201,225] Up to half of treated dogs experience an improvement in blood glucose levels, although some may become refractory to treatment. The recommended dosage is 10 to 50 μg given subcutaneously every 8 to 12 hours.[201] Streptozotocin is a nitrosourea alkylating agent that is directly cytotoxic to pancreatic beta cells. Its use in dogs historically has been limited by its nephrotoxicity, but a recent study indicates that streptozotocin can be safely administered using a 7-hour intensive saline diuresis protocol.[230] Preliminary results indicate that individual dogs may benefit from streptozotocin therapy, although overall improvement in the duration of euglycemia or survival has not been demonstrated in dogs with metastatic insulinomas.[230]

Dogs with insulinoma that undergo exploratory laparotomy followed by as-needed medical management are more likely to become euglycemic, remain euglycemic for longer periods, and have longer survival times compared to dogs managed only medically.[207] Even if measurable disease cannot be completely excised, any reduction in tumor burden may improve the efficacy of subsequent medical management. For dogs that undergo surgery, the long-term prognosis depends on the clinical stage of disease (Figures 24-9 and 24-10). Dogs without regional lymph node or distant metastasis at the time of surgery remain euglycemic for a median of 14 months, compared to approximately 2 months if metastatic disease is identified.[206] The median survival time is about 18 months for dogs without metastasis, 7 to 9 months for those with metastasis.[206]

Figure 24-9

Disease-free (i.e., normoglycemia) interval after surgery in dogs with insulin-secreting islet cell tumors based on clinical stage. Tumor that appears to be confined to the pancreas is classified as clinical stage 1 (T_1, N_0, M_0). Clinical stage 2 denotes spread to regional lymph nodes without any distant metastasis (T_1, N_1, M_0). Clinical stage 3 denotes distant metastasis with or without spread to local lymph nodes (T_1, N_1, M_1, or N_0, M_1). *(From Caywood DD, Klausner JS, O'Leary TP: J Am Vet Med Assoc 188:60-64, 1986.)*

Figure 24-10

Survival curve after surgery in dogs with insulin-secreting islet cell tumors based on clinical stage. Dogs with metastasis of the tumor beyond the regional lymph nodes (clinical stage 3 [T_1, N_0, M_1, or N_1, M_1]) have a significantly shorter survival time. However, dogs with metastasis only to regional lymph nodes (clinical stage 2 [T_1, N_1, M_0]) appear to have a survival time similar to that for dogs with tumor that appears to be confined to the pancreas (clinical stage 1 [T_1, N_0, M_0]) at the time of surgery. *(From Caywood DD, Klausner JS, O'Leary TP: J Am Vet Med Assoc 188:60-64, 1986.)*

Beta-Cell Tumors in Ferrets

Insulinomas are the most common tumor seen in ferrets, accounting for almost 25% of all tumors diagnosed in this species.[86] The median age at initial diagnosis is 5 years (range, 2 to 7 years). Ferrets most commonly show lethargy, weakness, difficulty rousing from sleep, and collapse.[209-211] Seizures occur infrequently. Ptyalism is exhibited by a significant number of ferrets with insulinomas but has not been reported in dogs or cats. More than half of ferrets with insulinomas have concurrent adrenocortical tumors and therefore may also exhibit clinical signs associated with excessive levels of sex hormones (see earlier discussion of adrenocortical tumors in ferrets).

A tentative diagnosis is made based on clinical signs and documentation of hypoglycemia. The serum insulin concentration can be measured, but it is important that the clinician make sure the assay has been validated for ferrets.[210] Up to 20% of affected ferrets have a normal serum insulin concentration. Imaging studies are of little benefit.

Medical management of ferrets with insulinoma includes *ad libitum* feeding and prednisone (0.5 to 1 mg/kg/day PO), with or without diazoxide (10 to 60 mg/kg/day PO) and octreotide (1 to 2 μg/kg given subcutaneously every 6 to 12 hours).[209-211] In one study of 10 ferrets managed medically, euglycemia was maintained for 0 to 2 months.[211] However, the survival time ranged from 1 to 9 months, which indicates that ferrets can live with hypoglycemia for extended periods.

Surgery has met with variable success because most ferrets have multiple pancreatic nodules. Even though metastasis is rare, in one study four of 27 ferrets that underwent pancreatic nodulectomy remained hypoglycemic postoperatively, and recurrence of hypoglycemia was documented in 22 of the remaining 23 ferrets.[211] The mean disease-free interval was 8 months (range, 0 to 18 months), and the mean survival time with supportive medical management was 15 months (range, 3 to 28 months). The best outcome was obtained when pancreatic nodulectomy was combined with partial pancreatectomy that included removal of all visible nodules plus the 25% to 50% of the pancreas that contained most of the nodules.[211] With this technique, one of 29 ferrets remained hypoglycemic postoperatively. Hypoglycemia recurred in most of the ferrets, but the mean disease-free interval improved to 12 months (range, 0 to 23 months) and the mean survival time to 22 months (range, 7 to 33 months).

GASTRINOMA

Gastrinomas are neuroendocrine tumors that secrete excessive amounts of gastrin. Zollinger-Ellison syndrome refers to the triad of a non-beta-cell neuroendocrine tumor in the pancreas, hypergastrinemia, and gastrointestinal ulceration. Gastrinomas are rare, with approximately 24 cases reported in dogs and 4 in cats.[231-236] Almost all reported gastrinomas were identified in the pancreas, although tumors have also been identified in peripancreatic lymph nodes (2 dogs) and the mesentery (1 dog). In contrast, approximately 40-50% of gastrinomas in people arise in the duodenum, 30-40% in the pancreas, 10-15% in lymph nodes, and <5% in a variety of ectopic locations.[216] While gastrin-producing G cells normally exist in the duodenum, they are not present in the pancreas. The cell of origin for primary pancreatic gastrinomas is not known, but D cells (which normally secrete somatostatin) are the most likely candidate.[231] Gastrinomas are highly metastatic, with involvement of the liver, regional lymph nodes, spleen, and/or mesentery identified in 70% of dogs and cats at the time of initial diagnosis.[231-236]

Dogs were 3-12 years old at presentation; cats were 10-12 years old.[231,232] No obvious breed or gender predilections have been identified. Clinical signs are referable to severe gastric acid hypersecretion.[231-236] The most common clinical signs are vomiting, weight loss, anorexia, and diarrhea. Gastrointestinal ulceration was identified in 80% of affected dogs and cats and some of these were perforated. Additional clinical signs associated with gastrointestinal ulceration include hematemesis, melena, hematochezia, and abdominal pain.

Gastrinoma should be suspected in any dog or cat with severe gastrointestinal signs. Baseline biochemical analysis (CBC, chemistry panel, urinalysis), abdominal ultrasound, and endoscopy typically reveal nonspecific abnormalities associated with gastrointestinal ulceration. Primary pancreatic tumors are almost invariably too small to be detected with ultrasound, although metastasis to regional lymph nodes or the liver may be visualized.[234] Scintigraphy using radiolabeled pentetreotide successfully identified a gastrinoma in a dog.[233] A tentative diagnosis is usually made by documenting a markedly elevated fasting serum gastrin concentration. Gastrin-provocative tests, including the secretin stimulation test and the calcium challenge test, are used in humans with borderline fasting serum gastrin levels.[231,232]

Exploratory laparotomy is recommended for dogs and cats suspected to have a gastrinoma. Even though 70% of dogs and cats have visible metastasis at the time of initial diagnosis, surgical debulking will reduce gastrin secretory capacity and enhance the efficacy of medical therapy.[232] In addition, deep or perforated gastrointestinal ulcers can be identified and excised. Long-term medical management includes the use of omeprazole, H_2-receptor antagonists, and sucralfate.[236] Octreotide has also been used in 2 dogs with success.[233,237] Survival times for dogs and cats with gastrinoma are typically less than 8 months.[231,232]

OTHER GASTROINTESTINAL ENDOCRINE TUMORS (APUDOMAS)

A variety of other gastrointestinal endocrine tumors exist. Many peptide-secreting endocrine cells, including those in the gastrointestinal tract, have the ability to synthesize and metabolize biogenic amines. These cells are called APUD cells (Amine Precursor Uptake and Decarboxylation), and tumors derived from these cells are called APUDomas. The APUDomas most commonly diagnosed in dogs include insulinomas, pheochromocytomas, parathyroid tumors, and medullary thyroid tumors (see above). Rarely diagnosed APUDomas include gastrinomas, glucagonomas (which are associated with superficial necrolytic dermatitis), pancreatic polypeptide-producing tumors, and carcinoids.[231,232,238-242]

MULTIPLE ENDOCRINE NEOPLASIA

MEN syndromes are heritable syndromes in humans that are characterized by the concurrent development of specific subsets of endocrine tumors.[97,243] Interestingly, most of the tumors inherited as part of MEN are APUDomas. MEN-1 results from germline mutations that inactivate the tumor suppressor gene menin.[243] The most common endocrine tumors associated with MEN-1 are parathyroid tumors, neuroendocrine tumors of the pancreas or duodenum (including gastrinomas and insulinomas), and pituitary tumors (prolactinomas, GH-secreting tumors and, rarely, ACTH-secreting tumors). MEN-2 includes MEN-2A and MEN-2B. Both syndromes are caused by autosomal dominant activating mutations in the receptor tyrosine kinase RET.[243] MEN-2A is most commonly associated with mutations involving the cysteine-rich extracellular domain, whereas MEN-2B usually results from mutations in the tyrosine kinase catalytic core. Almost all human patients with either MEN-2A or MEN-2B develop multifocal and bilateral medullary thyroid carcinoma, and up to half of affected people also develop pheochromocytomas. Patients with MEN-2A may also develop parathyroid tumors. Patients with MEN-2B all develop neural gangliomas, particularly in the mucosa of the digestive tract, conjunctiva, lips, and tongue.

Dogs occasionally are diagnosed with multiple endocrine tumors.[97,114,244-247] Most of these dogs only loosely fit the clinical criteria for the MEN syndromes described in humans, however eight dogs have been concurrently diagnosed with medullary thyroid carcinoma, pheochromocytoma, and parathyroid adenoma, consistent with MEN-2A.[114,244] Ferrets often are concurrently diagnosed with insulinomas and adrenocortical tumors.[87,211] Genetic analysis has not been performed in dogs or ferrets, and currently it is not known whether multiple tumors arise from underlying germline defects or are merely coincidental. Regardless, all dogs, cats, and ferrets diagnosed with an endocrine tumor should be thoroughly evaluated for additional endocrine tumors.

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Tumors of the Female Reproductive System

Mary K. Klein

OVARIAN TUMORS

Incidence

Ovarian tumors are uncommon in dogs and cats. Their true incidence is unknown, because most reports in the literature are based largely on necropsy surveys and biopsy submissions. Nevertheless, the reported incidence in the intact female dog is 6.25%[1] (0.5% to 1.2% of all canine tumors).[2-4] Incidence rates in the cat range from 0.7% to 3.6%.[5] The low rates in both species undoubtedly are biased by the large segment of the population that is surgically neutered at an early age.

Three general categories of ovarian tumors are described according to cell of origin: epithelial cell, germ cell, and sex cord stromal cell (Table 25-1). Epithelial tumors have been reported in dogs ranging in age from 4 to 15 years (median age, approximately 10 years).[3,4,6] Granulosa cell tumors, the most commonly identified sex cord stromal tumor, have been reported in dogs ranging in age from 14 months to 16 years.[3,4,6-8] In one study, 10 of 13 bitches with this tumor were nulliparous.[1] Teratomas, which arise from germ cells, have been reported in dogs ranging in age from 20 months to 9 years (most animals were 6 years old or younger).[6,9-12] In one series, pointers were found to be at increased risk for epithelial tumors, and English bulldogs were at increased risk for granulosa-theca cell tumors.[13] Boxers, German shepherds, and Yorkshire terriers were most commonly affected in a recent study.[4] Ovarian tumors have been reported in cats ranging from 2 months to 20 years of age (mean age, 6.7 years).[11,14,15]

Pathology and Natural Behavior

Canine ovarian tumors

The characteristics of the three general categories of ovarian tumors in dogs are outlined in Table 25-1. Epithelial and sex cord stromal tumors account for most recorded cases (80% to 90%).* Mesenchymal tumors,

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*References 3, 6, 7, 13, 16, and 17.

including primary ovarian hemangiosarcoma and fibromas (associated with prolonged mibolerone exposure), have been reported.[4,18]

Epithelial cell tumors

Epithelial cell tumors include papillary adenomas, papillary adenocarcinomas, cystadenomas, and undifferentiated carcinomas. These tumor types account for 40% to 50% of reported canine ovarian neoplasms.[3,6,16,17]

Papillary adenomas and adenocarcinomas can be bilateral.[6,7,16] Differentiation of the two forms can be difficult and is based on size, mitotic index, invasion into ovarian stroma, and extension into the ovarian bursa and adjacent peritoneum.[7,17] Papillary adenocarcinoma often is associated with widespread peritoneal implantation and the formation of a malignant effusion. Malignant effusions may develop by several mechanisms: edema in the ovarian tumor may cause leakage of fluid through the tumor capsule; the tumor may exfoliate cells, resulting in transcoelomic metastatic implants that exert pressure and obstruct peritoneal and diaphragmatic lymphatics; and secretion from metastatic peritoneal implants can occur.[2,19]

Papillary adenocarcinomas have been noted to metastasize to the renal and paraaortic lymph nodes, omentum, liver, and lungs.[3]

Cystadenomas appear to originate from the rete ovarii, are generally unilateral, and consist of multiple, thin-walled cysts that contain a clear, watery fluid.[7,16]

The term *undifferentiated carcinoma* is used to denote a tumor in which the embryonic morphology and absence of hormonal secretion preclude identification of a specific epithelial cell of origin.

Germ cell tumors

The primordial germ cells of the ovary are thought to be the origin of dysgerminomas, teratomas, and teratocarcinomas. Germ cell tumors account for 6% to 12% of canine ovarian tumors.[3,6] Dysgerminomas arise from undifferentiated germ cells and therefore consist of a relatively uniform population of cells resembling

TABLE 25-1 *Classification of Canine Ovarian Tumors*

	Epithelial Cell Tumors	Germ Cell Tumors	Sex Cord Stromal Tumors
Histologic classifications	Papillary adenoma Papillary adenocarcinoma Cystadenoma Undifferentiated carcinoma	Dysgerminoma Teratocarcinoma Teratoma	Thecoma Luteoma Granulosa cell tumor
Percentage of cases	40% to 50%	6% to 20%	35% to 50%
Bilateral incidence	Occasional	Rare	Rare
Functional incidence	Rare	Rare	~50%
Metastatic rate in malignant classification	~50%	~50%	<20%

ovarian primordial germ cells. They so closely resemble their testicular counterparts that they have been referred to as *ovarian seminomas*.[3,7,16,20] Bilateral dysgerminomas have been reported; however, most are unilateral.[21,22] Dysgerminomas grow by expansion and have a reported metastatic rate of 10% to 30%.[3,10,16,21,22] The most common metastatic site is the abdominal lymph nodes; however, involvement of the liver, kidney, omentum, pancreas, and adrenal glands has been reported.[3,7,10,21,22]

Teratomas are composed of germ cells that undergo differentiation into two or more germinal cell layers. Any combination of ectodermal, mesodermal, and endodermal tissues can be seen. These tissues generally are well differentiated histologically. Teratocarcinomas have both mature elements and undifferentiated elements resembling those of the embryo. A cumulative review of the literature revealed a 32% metastatic rate in canine teratomas/teratocarcinomas. Metastases were noted in multiple abdominal sites and in the lungs, anterior mediastinum, and bone. The metastatic lesions were noted to be composed predominantly of undifferentiated elements.

Sex cord stromal tumors

The most common sex cord stromal tumor is the granulosa cell tumor, which accounts for approximately 50% of ovarian tumors in several reviews.[1,3,6,7,16] Because sex cord stromal tumors arise from the specialized gonadal stroma of the ovary, which is responsible for estrogen and progesterone production, all these tumors have the potential to elaborate steroid hormones.

Granulosa cell tumors usually are unilateral and tend to be firm and lobulated, although cysts are commonly apparent on cross section. These tumors can grow quite large.[3,6] Sertoli-Leydig cell tumors can occur bilaterally.[6] Up to 20% of granulosa cell tumors demonstrate malignant behavior in the dog, with metastasis to the

sublumbar lymph nodes, liver, pancreas, and lung reported, as well as peritoneal carcinomatosis.*

Thecomas generally are benign in their behavior, growing by expansion without metastasis.[16,17] Luteomas are rare but have been reported in the dog; they are considered benign.[16,17,23] Alpha-inhibin, a gonadal glycopeptide known to be a feedback inhibitor of pituitary secretion of follicle-stimulating hormone (FSH), is a useful histogenetic marker of granulosa cell tumors and thecomas.[24]

Tumorlike conditions and metastatic involvement

Numerous turmorlike conditions can exist in the ovaries, and these often must be differentiated histologically. Ovarian cysts, which are common in the dog, can be very large and easily confused with a neoplastic process.[1] Paraovarian cysts, which arise from mesonephric tubules, can be single or multiple. Less common conditions include cystic rete tubules, vascular hematomas, and adenomatous hyperplasia of the rete ovarii.[16,17]

Although rare, metastasis to the ovary has been recorded in cases of mammary, intestinal, and pancreatic carcinoma and lymphoma.[1,16]

Feline ovarian tumors

Feline ovarian tumors also are defined as epithelial, germ cell, or sex cord stromal tumors. Sex cord stromal tumors are by far the most common, accounting for at least 50% of reported cases.[25,26] More than half of granulosa cell tumors in cats are malignant.[14,20,25-27] As is true in the dog, these tumors often show evidence of hormonally induced changes and are most commonly unilateral.[14,25,26] Reported metastatic sites include the

*References 2, 3, 6-8, 16, 17, and 20.

peritoneum, lumbar lymph nodes, omentum, diaphragm, kidney, spleen, liver, and lungs.[14,26,27]

In one series of 14 sex cord stromal tumors, the author classified five as interstitial gland tumors (luteomas). All were benign in behavior.[14] A similarly described tumor appeared to have virilizing effects in a 9-year-old cat.[26]

Approximately 15% of feline ovarian neoplasms are dysgerminomas.[14,21,25] In one study, two of six tumors were noted to be bilateral.[14] Dysgerminomas generally are considered to be hormonally inactive and slow to metastasize, yet metastasis has been reported in 20%[21] to 33%[14] of cases. These tumors tend to be encapsulated, smooth, dense tissue masses that can attain a large size within the ovary.[21,26] Teratomas are reported only rarely in cats, but they can demonstrate malignant behavior.[11,14,15,25,26]

Epithelial tumors are extremely rare in the cat.[14,25,26] Cystadenomas and ovarian adenocarcinomas have been reported.[14,26] Metastasis to the lungs, liver, and abdominal peritoneum was seen in one case of ovarian adenocarcinoma.[26]

Lymphomas and endometrial carcinomas have been noted to involve the ovaries secondarily in the cat.[25]

History and Clinical Signs

Canine ovarian tumors

The history and clinical signs of canine ovarian tumors vary, depending on the tissue of origin. Except for teratomas, which may be seen in young dogs, the tumors occur most often in middle-aged to older animals.[9-13]

Most epithelial cell tumors are asymptomatic until signs referable to a space-occupying mass occur.[7,19,20] Epithelial tumors may also manifest with malignant ascites.[4,7,19] Pleural effusions have been noted secondary to thoracic metastasis.[19] Granulosa cell tumors, dysgerminomas, and teratomas also have been associated with malignant abdominal effusions.[4]

Germ cell tumors have been associated with evidence of hormonal dysfunction but more commonly are associated with clinical signs of a space-occupying mass. Teratomas, in particular, can grow very large, and areas of calcification often are seen on routine abdominal radiographs.[7,9-11]

Sex cord stromal tumors are well documented as having the ability to produce steroid hormones.[3,6-8,16,20,23] Excessive estrogen production may lead to vulvar enlargement, sanguineous vulvar discharge, persistent estrus, alopecia, or aplastic pancytopenia. Excessive progesterone production leads to cystic endometrial hyperplasia/pyometra complex. Along with signs related to estrogen and progesterone production, one well-documented ovarian–steroid cell tumor resembling a luteoma has been associated with hyperadrenocorticism in a dog.[23] A hypoadrenal crisis occurred after surgical removal of the tumor. The clinical signs and pituitary/adrenal response to a dexamethasone injection normalized

1 month later. In rare cases, thecomas may produce estrogens, and luteomas can have secondary masculinizing effects. Sex cord stromal tumors may produce one or multiple hormones or none at all.[2,3,6,16,28] Whether functional or not, most sex cord stromal tumors are unilateral and large enough to be palpable by the time of presentation.[6-9,23]

Feline ovarian tumors

The granulosa cell tumor is the most often diagnosed feline ovarian tumor.[14,17,25,26] Clinical signs of hyperestrogenism are commonly reported, including persistent estrus, alopecia, and cystic or adenomatous hyperplasia of the endometrium.[14,25-27] Granulosa cell tumors generally are unilateral and large enough to be detected on palpation at presentation.[17,26,27] In at least one case, a functional androgenic interstitial gland tumor resulted in virilizing clinical signs.[26]

Dysgerminomas can be found bilaterally and may attain large size.[14,25,26] Signs of depression, vomiting, abdominal distension, and ascites usually are referable to the mass lesion.[25] The reported mean age of occurrence is 7 years.[26] Teratomas also have been reported on rare occasions, and the clinical signs noted were referable to their large size.[14,15,26]

Epithelial tumors are reported only rarely and can be found bilaterally.[14,26]

Diagnostic Techniques and Workup

No consistently abnormal laboratory findings are seen with ovarian tumors. An abdominal mass in an intact female animal with or without signs referable to the reproductive tract should place an ovarian tumor on the list of differentials. Small tumors are found close to the caudal pole of the kidney, whereas larger tumors are pendulous and mimic any midabdominal mass in location. The ultrasonographic appearance of canine ovarian tumor is well described.[29-31] Smaller tumors are easily identified as ovarian in origin, and the greater the solid proportion, the more likely the neoplasm is to be malignant. Cystic masses with regular contours are more often found in benign conditions.[29] Intravenous pyelography can help differentiate ovarian from renal masses. Cytologic evaluation of abdominal or pleural fluid often is suggestive of malignant effusions; signet-ring and rosette cellular patterns are suggestive of adenocarcinoma.[7,19] If functional neoplasia is suspected, evaluation of vaginal cytology for evidence of estrogen-induced cornification and/or serum progesterone measurement for values exceeding 2 mg/ml are indicated.[32] Radiographic evidence of calcification inside the mass is suggestive of a teratoma; however, definitive diagnosis rests with histopathologic evaluation of resected tissue. Transabdominal needle biopsies of the ovary are not recommended because of the propensity of many of these tumors to readily implant and grow on peritoneal

surfaces. Abdominal fluid may be safely aspirated to confirm malignant effusions. Thoracic radiographs should be evaluated for any evidence of metastatic disease, but they only rarely are positive at the time of diagnosis.

Therapy

Surgery remains the mainstay of treatment for ovarian tumors. A complete ovariohysterectomy is recommended, although oophorectomy alone is possible. Gentle handling of tissues to minimize transcoelomic tumor spread is warranted. Careful examination of all serosal surfaces, including the omentum and diaphragm, and removal or biopsy of any lesions suspected of metastatic disease are recommended for staging purposes.

Successful palliation with chemotherapy has been reported,[2,19] but no standard recommendations for therapy have been established. Intracavitary instillation of cisplatin has been beneficial for controlling malignant effusions.[33,34] Platinum-based combination regimens using the taxanes are the regimens of choice in human patients but have not been evaluated in the dog. Radiation therapy rarely is indicated or used because animals with disease confined to the ovary (amendable to radiation) usually are successfully treated with surgery.

Prognosis

Survival and outcome data are sparse for canine ovarian tumors. Survival times up to 4 years after removal of ovarian dysgerminomas and 6 years after removal of teratomas have been reported.[10] The prognosis for all ovarian tumors appears to be the same, regardless of histology. The prognosis is good when single tumors are completely excised at surgery. With any evidence of metastatic disease, the prognosis must be considered poor. Based on the human experience, chemotherapy seems to have the potential to lengthen survival times in canine patients with evidence of metastatic disease and should be considered.

Although numerous surgical descriptions exist, survival times after treatment are unavailable for feline ovarian tumor except for two cases. In one case, involving a teratoma, the cat was disease free 1 year after surgery. In the other case, involving a granulosa cell tumor, the patient was euthanized 5 months after surgery for suspected recurrence.[15,35] The prognosis likely is similar to that for other species; that is, reasonably good if solitary masses are completely excised, poor if evidence of metastatic disease exists.

Comparative Aspects

Ovarian tumors are the leading cause of death arising from gynecologic tumors.[36] Human and canine tumors share many characteristics:

- They have similar incidence rates.
- The incidence of epithelial tumors increases with age, and the incidence of teratomas is higher in a younger set of patients.
- Epithelial tumors show a tendency for transcoelomic metastasis.[1,19,36]

More than 90% of human ovarian tumors are epithelial in origin. By the time these patients seek care, most of them have advanced, disseminated disease; fewer than 30% have disease that is confined to the reproductive system at the time of diagnosis. Several prognostic factors have been elucidated in human clinical studies, including the histologic grade, stage of disease, extent of residual disease after surgery, and the presence of malignant ascites.[36]

Human ovarian epithelial tumors are well documented to be chemoresponsive, and the statistically significant improvement in 5-year survival rates over the past 3 decades is largely due to effective chemotherapy regimens and aggressive supportive care. Chemotherapeutic drugs that have demonstrated efficacy as single-agent treatments against human ovarian tumors include doxorubicin, platinum agents, taxol, 5-fluorouracil (5-FU), gemcitabine, and melphalan. As mentioned previously, platinum-based combination chemotherapy regimens, especially those using the taxanes, appear to have the best chance of obtaining a complete response in advanced ovarian carcinoma.[36]

Sex cord stromal tumors account for approximately 5% of cases. These tumors tend to be in the early stages when the patient seeks care, because they frequently are associated with hormonal effects that lead to early clinical signs. Granulosa cell tumors are the most common tumor diagnosed in this category. Overall survival is 85% to 90% when the tumor is diagnosed early.[36]

Germ cell tumors account for 2% to 3% of cases and most commonly are reported in women under 35 years of age. Dysgerminomas can occur bilaterally and are exquisitely sensitive to radiation therapy and chemotherapy. Teratomas generally are diagnosed between 10 and 20 years of age. Their prognosis is related to the relative amount of the most aggressive malignant component of the tumor.[36] Canine teratomas appear to be more malignant than their human counterparts.[37]

UTERINE TUMORS

Incidence

Uterine tumors are rare in both the dog and the cat; they account for 0.3% to 0.4% of all canine tumors[7,20,38,39] and 0.2% to 1.5% of tumors in the cat.[40-42] Middle-aged to older animals are most commonly affected, although uterine carcinoma has been reported in dogs as young

as 10 months.[43] No breed predilections have been reported in either species.[20,25,39]

Pathology and Natural Behavior

Most canine uterine tumors are mesenchymal in origin; leiomyomas account for 85% to 90% and leiomyosarcomas for 10% of mesenchymal tumors.[20,38] On rare occasions adenomas, adenocarcinomas, fibromas, fibrosarcomas, and the lipomas have been reported.[7,38,44,45]

Leiomyomas generally are noninvasive, nonmetastatic, and slow growing.[7] Grossly, they are difficult to distinguish from their malignant counterparts.[38,45] A syndrome characterized by multiple uterine leiomyomas, bilateral renal cystadenocarcinomas, and nodular dermatofibrosis has been characterized in German shepherds; this syndrome has been noted to have a hereditary component associated with a mutation in the canine Birt-Hogg-Dube (BHD) gene.[46,47]

In the cat, uterine adenocarcinomas account for most uterine tumors and arise from the endometrium.[25,48] Metastasis to the cerebrum, eyes, ovaries, adrenal glands, lungs, liver, kidneys, bladder, colon, diaphragm, and regional lymph nodes has been reported.[42,48] Other uterine neoplasms reported less often in the cat are leiomyomas, leiomyosarcomas, fibrosarcomas, lymphomas, fibromas, and lipomas.[25]

History and Clinical Signs

Canine leiomyomas and leiomyosarcomas rarely are associated with clinical signs, and many are incidental findings during necropsy or ovariohysterectomy. They can grow large enough to compress adjacent viscera, and vaginal discharge and pyometra may accompany malignant or benign uterine tumors.[7,38,39] Clinical signs secondary to metastatic disease may be the presenting complaint in cases of uterine and cervical carcinoma.[45,49]

Clinical signs may not be associated with feline uterine adenocarcinomas until the tumor becomes large. Vaginal discharge is common and can vary from purulent to mucoid to darkly hemorrhagic. Other reported clinical signs include abnormal estrous cycles, polydipsia, polyuria, vomiting, and abdominal distension.[25,48] Clinical signs may occur as a result of metastatic disease before any clinical indication of the primary tumor is seen.[48,50]

Diagnostic Techniques and Workup

No consistent laboratory abnormalities have been noted, and abdominal radiographs only confirm the presence of an abdominal or uterine mass. A definitive diagnosis usually is obtained through histologic examination of surgically excised specimens. Ultrasonography may further delineate the origin of the mass; however,

the uterus of cats and dogs generally cannot be visualized by this technique unless it contains fluid, a fetus, or a mass.

Therapy

A complete ovariohysterectomy is recommended, and attempts should be made to remove all tumors and metastatic foci. Chemotherapy and radiation therapy are largely untested in veterinary cases.

Prognosis

The prognosis for leiomyomas and other benign tumors is excellent, because surgery nearly always is curative. For leiomyosarcomas and other malignant tumors, the prognosis also is good if no evidence of metastatic disease is seen at the time of surgery and complete excision is possible. Because clinical signs rarely are evident until late in the course of disease, the prognosis should always be considered guarded until histopathologic examination and staging are complete. Nonresectable or metastatic disease warrants a grave prognosis.

Feline uterine adenocarcinomas have well-documented metastatic potential, therefore the prognosis for these tumors must be considered guarded.[25,44]

Comparative Aspects

Endometrial carcinomas account for 90% of human uterine tumors. Of affected individuals, 80% to 90% enjoy long-term survival because most have an early onset of symptoms. The median age at diagnosis is approximately 60 years, and the hallmark clinical sign is postmenopausal bleeding. Prognostic factors include the histologic grade, cell type, depth of myometrial invasion, and extension to the cervix. Ovariohysterectomy is the mainstay of therapy. High-risk cases may be treated adjuvantly with radiation. The long-term survival rate for uterine sarcomas after surgery alone is 60% to 70%.[51]

VAGINAL AND VULVAR TUMORS

Incidence

Vulvar and vaginal tumors account for 2.4% to 3% of canine neoplasms.[39,52] They are the second most common canine female reproductive tumor, after those of the mammary gland. Most of these tumors are benign, arise from smooth muscle (i.e., leiomyomas), and are found in intact female dogs 2 to 18 years of age (average age, 10.8 years).[20,39,52,53] The incidence of these tumors is higher in nulliparous bitches. One study found the boxer to be at increased risk over other breeds.[52]

Lipomas tend to occur in younger dogs ranging in age range from 1 to 8 years (mean age, 6.3 years).[39]

Incidence rates are not available for the cat. Leiomyomas and a fibroma have been reported.[25,54] Vaginal leiomyomas were noted in older intact queens.[25,54]

Pathology and Natural Behavior

As many as 86% of vulvar and vaginal tumors are reported to be benign smooth muscle tumors.[52,53] These are referred to as leiomyomas, fibroleiomyomas, fibromas, and polyps, and they vary only in the amount of connective tissue present. Because the clinical course of the tumor does not appear to be affected by histologic variations, these tumors can be considered collectively.[39,52] In endemic areas, transmissible venereal tumors (TVTs) are common (see Chapter 32, section C).

Most leiomyomas arise from the vestibule of the vulva rather than from the vagina.[39,52] Extraluminal and intraluminal forms have been described. The extraluminal form manifests as a slow-growing perineal mass. On cut section these tumors are gray to white to tan, well encapsulated, and poorly vascularized.[7,38,39]

Intraluminal tumors are attached to the vestibular or vaginal wall by a variably sized pedicle. They often are firm and ovoid, and although their mucosa generally is intact, ulceration may occur with exposure, irritation, or secondary infection. Intraluminal tumors can be multiple, suggesting a hormonal influence.[38,52,53] In one study, all pedunculated or polypoid tumors were found to be benign.[52]

Subjective data indicate that leiomyomas may be hormone dependent. In two studies, all dogs diagnosed with leiomyoma, fibroma, or polypoid tumors were intact.[52,53] The recurrence rate was 15% in dogs left intact after local resection, and no recurrence was seen in dogs that underwent ovariohysterectomy at that time. In another study, leiomyomas were not seen in bitches ovariectomized before 2 years of age.[39] Concurrent uterine, ovarian, and mammary gland changes, including cystic endometrial glandular hyperplasia, ovarian cysts, and mammary gland tumors, were present in approximately one third of cases.[39,53]

Other reported benign vaginal and vulvar tumors are lipomas, sebaceous adenomas, fibrous histiocytomas, benign melanomas, myxomas, and myxofibromas.[7,20,39,52]

The most common malignant tumor is leiomyosarcoma. Distant metastases have been reported.[39,52] Other tumors with malignant potential that have been reported include TVT, adenocarcinoma, squamous cell carcinoma, hemangiosarcoma, osteosarcoma, mast cell tumor, and epidermoid carcinoma.[7,20,38,39,52]

The labia of the vulva may be a site for any tumor associated with cutaneous tissues. Carcinomas arising from the bladder or urethra may also manifest as palpable vaginal masses near the urethral papilla.[53,55]

History and Clinical Signs

The duration of clinical signs tends to be longer with extraluminal tumors.[39] The presenting clinical sign generally is a slow-growing perineal mass (Figure 25-1). Intraluminal tumors, by virtue of their tendency for pedunculation, often appear externally when straining causes the mass to extrude through the vulvar lips, especially at the times of estrus (Figure 25-2). Most owners complain of a mass that "pops out" of the vulva. Clinical signs seen less frequently include vulvar bleeding or discharge, an enlarging vulvar mass, dysuria, hematuria, tenesmus, excessive vulvar licking, and dystocia. A greater proportion of malignancy is noted in vaginal and vulvar tumors that demonstrate frequent recurrence and lack of pedunculation.[38,39,52,53]

A lipoma manifests as a slow-growing mass that eventually impinges upon adjacent structures. These tumors can arise from the perivascular and perivaginal fat and lie within the pelvic canal. They may attach to the tuber ischium. All are reported to be well circumscribed and relatively avascular.[38,39]

In cats with leiomyoma, the tumor masses are firm, and the presenting clinical sign of constipation occurs secondary to dorsal compression of the rectum and dilation of the colon anterior to the tumor. In one report, one cat also had cystic ovaries and a mammary adenocarcinoma.[54]

Diagnostic Techniques and Workup

Although the signalment (i.e., aged, intact female), combined with the location and gross appearance of the tumor, is suggestive, definitive diagnosis is obtained through histopathologic examination of excised tissue. Vaginoscopic examination, retrograde vaginography, or urethrocystography may help delineate a suspect mass. Aspiration cytology may be helpful for further identifying the origin of the mass and the possibility of malignancy. Caudal abdominal radiographs may be indicated for an extraluminal mass with extension cranially. Ultrasonography or advanced imaging also may be helpful (e.g., computed tomography [CT], magnetic resonance imaging [MRI]).

Therapy

In light of the evidence of hormonal dependence and the high incidence of disease in aging bitches with an intact reproductive system, an ovariohysterectomy at the time of treatment appears to be a prudent course of action. This also permits examination of the abdominal organs for evidence of metastatic disease. Conservative surgical excision combined with ovariohysterectomy usually is curative for benign tumors. Intraluminal tumors can be removed easily by placing one or more

A   B
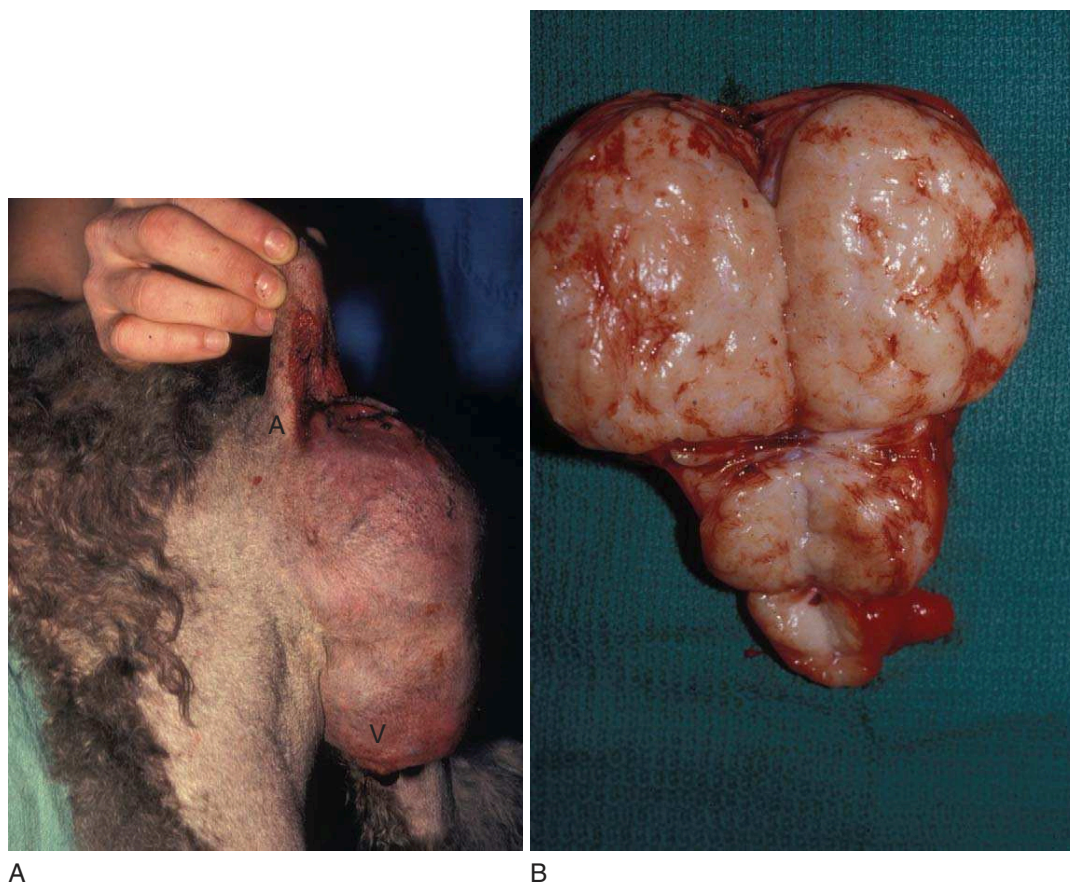
Figure 25-1

A, Slow-growing perineal mass typical of an extraluminal leiomyoma. **B,** A midline perineal incision allowed the removal of this well-encapsulated mass sectioned longitudinally from **A**. Histologic examination confirmed a benign leiomyoma of the vagina. *A,* Anus; *V,* vulva.

Figure 25-2

Pedunculated intraluminal vaginal leiomyoma. This tumor often prolapses during estrus, and the exposed surface may ulcerate when traumatized. Treatment in this case included removal by simple ligation of the pedicle and concomitant ovariohysterectomy.

transfixing sutures in the pedicle. Wide removal is not necessary if ovariohysterectomy is performed, even though these tumors probably arise in the smooth muscle of the vaginal wall. If the pedicle or urethral papilla cannot be visualized adequately, a dorsal episiotomy ensures safe resection and allows visualization of the urethra.

Surgical removal of extraluminal tumors also can be readily accomplished through a dorsal episiotomy. Because these tumors tend to be well encapsulated and poorly vascularized, blunt dissection generally removes them entirely. On rare occasions, a perineal approach or pelvic split may be required. Catheterization of the urethra helps prevent accidental damage to that structure. If the primary tumor and metastatic foci cannot be completely excised, local radiation therapy may be beneficial.[38] Malignant, infiltrative vaginal neoplasms can be addressed with complete vulvovaginectomy and perineal urethrostomy in carefully selected cases.[56]

Cases with multifocal disease can be managed with ovariohysterectomy (OHE) alone, with the hope that stable disease or regression can be obtained with hormone ablation.

Prognosis

Surgery for benign lesions is nearly always curative, and vulvovaginal tumors are rarely identified as a cause of death. Many are incidental findings at necropsy. The prognosis for adenocarcinomas and squamous cell carcinomas generally is poor because of these tumors' high rates of local recurrence and metastasis.[38,52] Surgery with OHE was curative in the feline leiomyoma cases reported.[54]

Comparative Aspects

Vaginal cancer is seen in elderly women (70% to 80% are over age 60), except for women secondarily exposed to maternal diethylstilbestrol, in whom the cancer generally develops before the age of 40. Squamous cell carcinomas comprise 80% to 90% of cases; 5% to 10% are adenocarcinomas, 3% are melanomas, and 3% are sarcomas. The prognosis depends on the clinical stage at the time of diagnosis. Carcinoma of the cervix is associated with exposure to the human papillomavirus (HPV), and 80% to 90% of these tumors are squamous cell carcinomas.[57]

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CANINE MAMMARY TUMORS

Mammary neoplasms are among the most common tumors of the female dog. Estimates of lifetime risk for malignant tumors vary from 2% to more than 20%,[1,2] a risk assessed to be exceeded twofold to fivefold by that of benign mammary tumors. Two studies of large populations of dogs have recently reported incidence information. The first study, involving a defined population of insured dogs in the United Kingdom, reported a standardized incidence rate for mammary tumors of 205 per 100,000 dogs per year.[3] The second study surveyed a population of more than 80,000 insured female dogs in Sweden and found an overall incidence for any mammary tumor of 111 per 10,000 dog years at risk.[4] This study also found that the incidence increased with age; at age 6 years, it was 1%; at age 8 years, 6%; and at age 10 years, 13%.[4] The incidence of both benign and malignant tumors in specific populations of dogs correlates with life expectancy and is strongly reduced by the practice of ovariohysterectomy in young dogs, which is common in the United States but not encouraged in some European countries. The incidence is increased by the use of injectable progestins to prevent estrus. The risk in male dogs is 1% or less of that in female dogs.

The median age of tumor manifestation is 10 to 11 years of age, with rare occurrence in dogs younger than 4 years old. Several spaniel breeds and, according to some studies, the poodle and dachshund seem to be predisposed to the condition.[5-7] A recent report, however, suggested that the incidence of malignant tumors was different in small breed dogs compared with large breed dogs.[8] In this study, of 101 tumors (60 small breed, 40 other), 25% of the small breed dogs had histologically malignant tumors compared with 58% of the large breed patients.

The development of mammary tumors in the dog is clearly hormone dependent. Compared to the risk in intact dogs, the risk for malignant tumors in dogs spayed before the first estrus is 0.05%; after the first estrus, it is 8%; and it rises to 26% if the dog is spayed after the second estrus.[9] Later spaying does not reduce the risk for malignant tumors, although the risk for benign tumors seems to be reduced by ovariectomy even at a later age.[10] In a retrospective study of 137 dogs with malignant mammary carcinomas, dogs spayed within 2 years of the development of the malignant tumor showed a survival advantage over dogs intact or spayed 2 years or longer before mastectomy.[11]

A protective effect of (early) pregnancy, well-known in humans, has not been demonstrated.[9] Most likely, the tumor-enhancing effect of ovarian steroids, predominantly progesterone, is brought about by their mitogenic activity in mammary cells, exerted after they bind to their respective receptors. Normal mammary gland tissue contains both estrogen receptors (ERs) and progesterone receptors (PRs) receptors (as do most benign tumors), often at increased level. In contrast, carcinomas, devoid of remnants of normal mammary epithelium, contain ERs and PRs in a decreased number of cases, with rare occurrence in metastases.[12-16] In a study by Millanta and colleagues,[17] 85 mammary lesions (28 dysplasias, 21 benign lesions, and 36 carcinomas [4 *in situ*, and 32 invasive]) from 47 dogs were evaluated by means of immunohistochemistry for ER and PR expression. Ten normal mammary tissues were used for comparison. The researchers found that 100% of the normal and dysplastic tissues and 95% of the benign tissues tested positive for ERs. Of the invasive carcinomas, 92% showed some ER staining, although only 62% had strong nuclear staining. No significant difference in quantitative ER expression was found between normal, dysplastic, benign, and *in situ* lesions, whereas the ER expression in invasive carcinomas was significantly lower. In this same study, PR expression was significantly lower in both benign and malignant lesions compared with healthy tissue.[17]

In another report, 228 tumors (155 malignant, 73 benign) from 100 dogs were evaluated by immunohistochemistry for ER and PR expression.[18] In all tumors, one or both receptors were detected in 76% of samples (96% of benign samples, 66% of malignant

samples). In seven cases of lymph node metastasis, both the primary tumor and the node were considered ER and PR negative. Overall, this pattern suggests a loss of steroid dependency for malignant mammary tumors. Prolactin, an important hormone for the development of mammary tissue, has been examined in tissue and serum from canine patients with both benign and malignant mammary tumors.[19] Prolactin levels were significantly higher in the tissues and serum from malignant tumors than in normal mammary tissue.

It is well known that in the female dog, progesterone or synthetic progestins such as chlormadinone acetate (CMA) or medroxyprogesterone acetate (MPA) induce full lobuloalveolar development of the mammary gland, with hyperplasia of secretory and myoepithelial elements, whereas estradiol stimulates ductal growth. However, prolonged administration of estrogens has not been shown to increase the incidence of mammary tumors in dogs. Conversely, progesterone administered to young female beagles mainly leads to the development of benign nodules. The risk for malignant tumors becomes higher after long-term experimental administration of estrogens combined with progestins at high dosage or if drugs are used that have a combined progestagenic-estrogenic activity.[20] Injectable progestins used to prevent estrus in dogs have been shown to increase the incidence of benign tumors but not malignant tumors,[10] although an increased incidence of the latter also has been reported.[21] Administration of progestins to dogs also has been shown to increase the secretion of growth hormone (GH). This progestin-induced GH gene expression was found to originate from normal mammary gland epithelium.[22] It is not known whether the progestin-induced GH acts as an intermediate in the progestin-stimulated development of canine mammary tumors.

Parallel to the increase in GH production induced in the dog by progestins, a rise in the blood levels of insulin-like growth factor I (IGF-I) and IGF-II has been shown to occur,[23] which may stimulate mammary cell proliferation. Hormonally controlled and autonomous expression of growth factors and their receptors also may influence mammary tumorigenesis, making it a highly complex process.

Many genes are involved in the normal transmission of growth-promoting signals from the cell surface to the nucleus (e.g., *ras*). The *ras* proteins tend to be overexpressed in many human tumors, but studies of canine mammary tumors using polymerase chain reaction (PCR) analyses have failed to reveal expression of *ras* genes.[24,25] Regarding oncogenes expressing for the receptor of growth factors, one study found overexpression of messenger ribonucleic acid (mRNA) for c-erbB-2 (also called *c-neu*) in most canine malignant (but not benign) mammary tumors,[26] whereas another study found that its protein was expressed more often in

benign tumors (50%) than in malignant ones (19%).[27] Recently Dutra and coworkers[28] reported on c-erbB-2 protein expression in 48 malignant canine mammary tumors using the commercially available HercepTest system, which is an immunohistochemistry-based assay approved for use in human breast cancers. They found that although none of the 22 benign tumors overexpressed c-erbB-2, 35.4% of malignant tumors did.[28] Standardization of methodology is necessary to allow proper interpretation of results and to elucidate the role of c-erbB-2 in canine mammary neoplasia.

The p53 tumor suppressor gene is the most frequently mutated gene in human cancer. Recent studies in canine mammary cancer found that three of 10[29] and six of 40[30] primary cancers contained p53 mutations, with one dog carrying a germ-line mutation.[29,30] In another study, 17% of 69 canine mammary carcinomas assayed showed a p53 gene mutation, and multivariate analysis indicated that this mutation conferred an increased risk of recurrence and death from mammary tumor.[31] Alterations of a second tumor suppressor gene, BRCA1, which is responsible for part of hereditary human breast cancers, has been seen in some canine mammary cancers.[32] However, the extent to which specific genetic alterations contribute to the pathogenesis of canine mammary tumors still needs to be resolved. Gross abnormalities in the nuclear deoxyribonucleic acid (DNA) content (DNA aneuploidy), as detected with flow cytometry, have been found in 50% to 60% of primary cancers, occurring as either an increase or a decrease in DNA content.[33–35] Also, 15% to 25% of benign mammary tumors were aneuploid, possibly reflecting the potential to progress to malignancy.[33-35]

Other factors that may play a role in the progression of canine mammary tumors have been evaluated, including expression of adhesion and gap junction proteins such as E-cadherin, connexins, and paxillin. In general, the more invasive, proliferative, and aggressive a tumor was histologically, the less localized and intense the protein expression was, indicating a shift that favored increased cell mobility.[36-38] Apoptotic pathways also have been explored as a contributor to mammary carcinogenesis. In a recent study, human and canine tumors were found to have increased patterns of expression of antiapoptotic proteins (bcl-2, bcl-Xl) and a significantly decreased expression of proapoptotic proteins (Bax, caspase 8 and 3).[39]

Induction of cyclooxyegenase-2 (COX-2) enzymes, thought to be involved in human breast carcinogenesis, also has been evaluated in normal, benign, and malignant canine mammary tissue. Dore and colleagues[40] found that COX-2 was not expressed in normal tissues but showed increased expression in benign (24%) and malignant (56%) tumors, indicating a possible role in tumorigenesis.[40] An association also has been shown between COX-2 expression and tumor histologic subtype.[41]

Experiments in rodents and epidemiologic studies in humans have shown that a high-fat diet and obesity increase the risk of mammary cancer. A recent study in pet dogs in the United States found that among spayed dogs, the risk of developing mammary cancer was reduced if the dogs were thin at 9 to 12 months of age.[42] A study in Spain made a similar observation, that obesity at 1 year of age was a risk factor for the development of benign and malignant mammary tumors without consideration of ovariectomy.[43] Somewhat surprisingly, the intake of homemade meals (compared with commercial foods) also was related to an increased risk. Nutritional factors, therefore, may play a role in canine mammary tumor development.

Pathology and Natural Behavior

Based on material from veterinary practices submitted for histologic diagnosis, 41% to 53% of mammary tumors that occur in the bitch are considered malignant.[44,45] Histologic evidence of malignancy does not invariably imply a malignant clinical course. Also, marked variation in histologic appearance can occur within the same tumor mass.

Most malignant mammary tumors are classified as epithelial tumors or carcinomas. Pure sarcomas (fibrosarcoma, osteosarcoma, sarcoma of another type) represent a minority. Whether sarcomas arise from myoepithelial tissue that has undergone neoplastic change or from the intralobular connective tissue is unclear. No evidence indicates that sarcomas arise from pre-existing, benign mixed cell tumors. Some malignant tumors are composed of cells that morphologically resemble the epithelial and connective tissue components (both of which are malignant); these tumors, which are uncommon, are called *carcinosarcomas* (in some systems, *malignant mixed tumor*). Benign tumors include simple/complex adenomas, fibroadenomas, and the relatively common benign mixed tumor, which has an epithelial component and a mesenchymal component of cartilage and/or bone and/or fat, both components possibly originating from a pluripotent stem cell.[46,47]

Some histologic classification systems are based on recognition of histologic tissue patterns thought to identify the histogenetic origin of the tumor cells. However, this may have little reference to disease behavior. The revised classification established by the World Health Organization (WHO) (Box 26-1), in an attempt to obtain a division of prognostic weight, is descriptive and subdivides carcinomas further into noninfiltrating, complex, or simple carcinomas (simple carcinomas are the tubulopapillary, solid, or anaplastic type). This subdivision is believed to rank the tumors by increasing potential for malignancy.

In a recent study covering the full life span of 672 intact female beagles, the multifocal nature of

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Box 26-1 | **Histologic Classification of Canine Mammary Tumors**

Malignant tumors
Noninfiltrating *(in situ)* carcinoma
Complex carcinoma
Simple carcinoma
  Tubulopapillary carcinoma
  Solid carcinoma
  Anaplastic carcinoma
Special types of carcinomas
  Spindle cell carcinoma
  Squamous cell carcinoma
  Mucinous carcinoma
  Lipid-rich carcinoma
Sarcoma
  Fibrosarcoma
  Osteosarcoma
  Other sarcomas
Carcinosarcoma
Carcinoma or sarcoma in benign tumor

Benign tumors
Adenoma
  Simple adenoma
  Complex adenoma
  Basaloid adenoma
Fibroadenoma
  Low-cellularity fibroadenoma
  High-cellularity fibroadenoma
Benign mixed tumor
Duct papilloma

From Misdorp W, Else R, Hellman E et al: Histologic classification of mammary tumors of the dog and cat. In World Health Organization international histological classification of tumors of domestic animals, series 2, vol 7, no 2, Washington, DC, 1999, Armed Forces Institute of Pathology.

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mammary neoplasia was illustrated, with 71% having at least one mammary neoplasm and 61% more than one tumor, accumulating to a total of 4755 neoplasms, including 1639 carcinomas.[48] Based on a histogenetic classification system, the investigators found that of the carcinomas, ductular carcinomas represented 19% and adenocarcinomas of other histogenetic origin accounted for the rest. Only 73 carcinomas were fatal, and ductular carcinomas were eight times more likely to result in fatalities than adenocarcinomas, even though the difference in total (regional plus distant) metastasis rate (46% versus 32%) was less. In this same study, true carcinosarcomas were diagnosed in only five dogs, all of which died of metastatic disease.

Another study of 356 female beagles determined a lifetime risk of 63% for the development of any mammary hyperplasia or tumor, and 23% for the development of a malignant tumor.[2] In this study the

metastatic rate was higher, reaching 77% in 72 dogs with carcinoma. Infiltration of the carcinoma into adjacent tissues was considered of greater prognostic importance than the histologic subtype.[2]

A different study applied a classification system adopted from a prognostic human pathologic staging system in 158 pet dogs with mammary cancer.[45] Carcinomas were assigned to one of four histologic grades: (1) lesions that were noninfiltrating (i.e., *in situ* carcinoma [grade 0]); (2) lesions that invaded the surrounding stroma but without identifiable vascular or lymphatic invasion (grade I); (3) lesions that showed vascular or lymphatic invasion and/or metastasis to regional lymph nodes (grade II); and (4) lesions that showed pathologic evidence of distant metastasis (grade III). The tumors were categorized further according to the degree of nuclear differentiation (poorly differentiated, moderately differentiated, or well differentiated). Both the grade and the degree of differentiation were found to be significantly related to tumor aggressiveness.[45]

Another grading scheme recently was reported to have prognostic significance by Karayannopoulou and colleagues.[49] In this study, a grade was determined for each case by scoring three different criteria: tubule formation, nuclear pleomorphism, and mitotic counts. Each criterion received a score ranging from 1 to 3, and the total score was used to describe a tumor as well differentiated (grade I), moderately differentiated (grade II), or poorly differentiated (grade III). Eighty-five cases of carcinoma were followed for 2 years after mastectomy. The authors found that patients with grade III tumors had a significantly worse survival rate than those with grade I or grade II tumors. They also found a 21-fold increased risk of death from the tumor with grade III lesions compared to grade I or grade II tumors.[49]

A specific designation is reserved for inflammatory carcinoma. In one study, 17% of 186 malignant mammary tumors were classified as inflammatory carcinoma.[50] Histologically, there is evidence of a poorly differentiated carcinoma with extensive evidence of both mononuclear and polymorphonuclear cellular infiltrates and often edema. Dermal lymphatic invasion also can be seen histologically. Clinically, these neoplasms grow and metastasize extremely rapidly and invade lymphatics in the skin, resulting in marked edema and inflammation (Figure 26-1).[51] In a study of 21 cases, all were found to be estrogen receptor (ER) negative.[52]

History and Signs

Mammary tumors manifest clinically as single or (in more than half of cases) multiple nodules within the mammary gland, developing simultaneously or subsequently. Multiplicity may be less common in bitches with a limited duration of exposure to ovarian steroids.

Tumors may be associated with the nipple or, more often, the glandular tissue itself. The dog has five pairs of glands, all of which can develop one or more benign or malignant tumors. Roughly 65% to 70% of canine tumors occur in glands 4 and 5, probably because of the greater volume of mammary tissue in these glands. In animals with benign mammary tumors, the tumor is small, well circumscribed, and firm on palpation. Clinical signs of malignancy include rapid growth, ill-defined boundaries, fixation to the skin or underlying tissues, and ulceration or inflammation (Figure 26-2). The presence of one or more of these signs may indicate an increased risk of an underlying malignant growth.

Figure 26-1

Bilateral inflammatory carcinoma with vaginal edema and edema of the leg in a dog. Vaginal cytology and fine-needle cytology of the popliteal lymph node were positive for cancer.
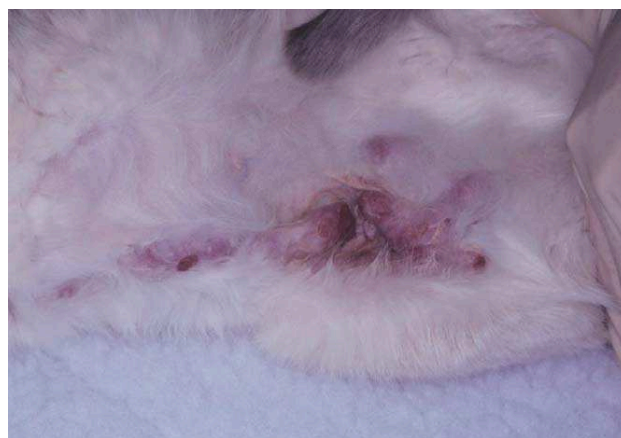
Figure 26-2

Inflammatory carcinoma in a dog, showing ulceration and nodular plaque formation.

As previously mentioned, the inflammatory carcinomas (ICs) have a unique clinical presentation. This tumor type should be suspected if a tumor is rapidly growing, affects multiple mammary glands and overlying skin, and is characterized by firmness, warmth, edema, erythema, thickening, and signs of pain. All or part of one or both mammary chains may be involved. Extensive lymphedema of a limb or limbs adjacent to this type of mammary cancer also may occur. Such edema is the result of occlusion of affected lymphatics, with accompanying retrograde growth down the limb. It is important to differentiate this type of malignancy from inflammatory mastitis, although the latter is an unusual condition in dogs. Inflammatory carcinomas tend to be quite firm and have a diffuse swelling, whereas mastitis tends to be more localized and usually is seen after estrus, pregnancy, or false pregnancy. Systemic signs often can accompany the diagnosis of IC. In one study, 94% of dogs with IC had generalized weakness, described as lack of energy, and decreased activity compared to only 18% of dogs with other mammary tumors.[50] Pain also was seen in all 33 dogs with IC (100%), compared to only 16% of dogs with other mammary tumors.

Carcinoma that metastasizes to the inguinal lymph nodes may enter the pudendal lymphatics and spread to the internal iliac nodes. Metastasis from the internal iliac nodes may be palpable and may cause pressure on and compression of the colon. The other common metastatic sites include the lungs, liver, kidneys and, less frequently, bone.[53]

Diagnostic Techniques and Workup

The diagnostic workup should include a thorough physical examination and a routine hematologic and serum chemistry profile to assess general health. A coagulogram may be indicated in dogs suspected of having inflammatory carcinoma because of the concurrent association with disseminated intravascular coagulation.[51,54] The first evaluation should reveal whether local or loco-regional disease is amenable to complete resection. The presence of an inflammatory carcinoma invariably is associated with an intravascular tumor, (microscopic) or metastatic disease, and rapid local or distant recurrence after attempted surgery. If malignancy cannot be excluded, thoracic radiographs in both the right and left lateral and ventrodorsal planes should be taken before surgery to evaluate the lungs and sternal lymph node for possible metastasis. In dogs, if the mammary tumors involve the caudal two glands, the sublumbar region should be evaluated for metastatic lymphadenopathy using caudal abdominal radiographs or ultrasonography. A rectal examination may reveal palpable evidence of internal iliac lymphadenopathy. Fine-needle aspiration with cytology to differentiate

benign from malignant tumors has been reported to be an insensitive method;[55] however, cytologic evaluation of the mass can rule out other lesions, such as inflammatory lesions or mast cell tumors. Fine-needle aspiration for cytologic evaluation may also be beneficial in the diagnosis of inflammatory carcinomas. If lymph node metastasis is suspected, cytology should be used to assess suspect nodes. The most definitive way to obtain a diagnosis is through tissue biopsy. Practitioners must keep in mind that a presurgical distinction between a benign and a malignant mammary tumor does not necessarily change the extent of the operative procedure.[55]

Clinical Staging

Accurate, precise staging is important before treatment is initiated (Box 26-2). The most important requirements of staging are to (1) evaluate the primary tumor, (2) evaluate the regional lymph nodes, and (3) attempt to identify any distant metastatic sites, including distant lymph nodes and the lungs. The most important features to note with the primary tumor are recent rapid growth, size, clinical evidence of invasiveness (fixation to skin or fascia), ulceration, and clinical evidence of inflammatory carcinoma. The most common sites of distant metastasis are the lungs; sublumbar, sternal, and prescapular lymph nodes; liver; and, rarely, bone.

Box 26-2 | **Staging of Canine Mammary Tumors**

Modified system

T—Primary tumor
T_1 <3 cm maximum diameter
T_2 3-5 cm maximum diameter
T_3 >5 cm maximum diameter

N—Regional lymph nodes
N_0 Histologic or cytologic—No metastasis
N_1 Histologic or cytologic—Metastasis present

M—Distant metastasis
M_0 No distant metastasis detected
M_1 Distant metastasis detected

Stages

I:	T_1	N_0	M_0
II:	T_2	N_0	M_0
III:	T_3	N_0	M_0
IV:	Any T	N_1	M_0
V:	Any T	Any N	M_1

Modified from Owen LN: Classification of tumors in domestic animals, Geneva, 1980, World Health Organization.

Therapy

Surgery remains the treatment of choice for all dogs with mammary gland tumors except those with inflammatory carcinomas or distant metastasis. The type of surgery depends on the extent of disease.

Surgical technique

The theoretical and practical pros and cons of radical versus local excision have been extensively debated.[56] In one prospective clinical trial of 144 dogs that compared simple mastectomy (i.e., removal of the affected gland or glands only) to chain mastectomy (i.e., chain resection on the affected side), no difference in recurrence rate and survival time was noted.[57] Proponents of chain mastectomy argue that it is the most likely procedure to remove all tumor (known or occult) and that it reduces future risk by reducing the volume of breast tissue at risk. Some also argue for more aggressive surgery based on the observation that some benign lesions, namely those with atypia, are linked with an increased risk for the development of invasive cancer.[45] These arguments may have more weight in relatively young, intact bitches. Those opposed to routine chain resection argue that it is too much surgery, when more than 50% of canine breast masses are benign; a more aggressive procedure can always be performed later on the 40% to 50% of patients with a malignant histology (especially if margins were incomplete); and chain mastectomy increases the morbidity, time, and expense of the treatment.

The primary goal of surgery is to remove all tumor by the simplest procedure, which should take into consideration possible extension of malignant lesions through mammary lymphatics to regional nodes. More radical surgery for localized lesions may lead to a lower risk for the development of new tumors in only a subset of dogs, mentioned previously; however, it does not inhibit the outgrowth of occult metastases of the tumor to be treated.

A variety of procedures can be used to remove canine breast tumors, and the choice of procedure is determined by the size of the tumor, whether it is fixed to surrounding tissue, the number of lesions, and the probability that a local cure can be achieved.

Lumpectomy (Nodulectomy). Lumpectomy, also called nodulectomy, is indicated for small (less than 0.5 cm), firm, superficial, nonfixed nodules, which usually are benign. *This procedure is not to be used for known malignant tumors.* The skin is incised, and the nodule is bluntly dissected from the breast tissue with a small rim of normal tissue surrounding the tumor nodule.

After resection the tumor is classified as benign or malignant, and the completeness of removal is evaluated. For benign lesions, even close and incomplete removal probably is adequate. If the lesion is small,

well circumscribed, and malignant, close but clean margins (1 to 2 cm) are acceptable. Incomplete resection of malignant tumors warrants more aggressive removal of the entire gland.

Mammectomy. Removal of one gland is indicated for lesions that are centrally located within the gland and larger than 1 cm and that show any degree of fixation to the skin or fascia. Skin and/or abdominal wall fascia should be removed with the mass, if involved.

Based on the individual patient's gland confluency, removing glands 4 and 5 as a unit or glands 1, 2, and 3 as a unit may be easier than extensively dividing normal mammary tissue. In other words, if the individual gland is a distinct anatomic unit, single gland removal is acceptable.

Regional Mastectomy. Regional mastectomy originally was proposed based on the known venous and lymphatic drainage of the mammary tissue.[58,59] In the dog, the mammary glands have been identified, depending on their position, as the cranial thoracic gland (gland 1), caudal thoracic gland (gland 2), cranial abdominal gland (gland 3), caudal abdominal gland (gland 4), and inguinal gland (gland 5). Lymphatic drainage from the mammary glands has been documented to the axillary, superficial inguinal, sublumbar, and cranial sternal nodes, depending on the gland involved.

The lymphatic drainage of canine mammary tumors is complex. Although lymphatic drainage can occur between all glands, a recent report indicated that major connections exist between glands 1 and 2 and between glands 4 and 5.[59] Mammary glands 1, 2, and 3 and occasionally 4 drain to axillary and cranial sternal lymph nodes. The superficial inguinal lymph nodes provide lymphatic drainage to mammary glands 3, 4, 5, and occasionally 2. The superficial inguinal lymph nodes have efferent drainage to the medial iliac lymph nodes, which then continue to the lumbar trunks and finally to the cisterna chyli. Based on the premise of lymphatic drainage, tumors involving glands 1, 2, or 3 should be removed en bloc. Similarly, tumors involving gland 4 or 5 should be removed en bloc, including the adjoining lymph nodes, which is always possible. The axillary lymph node is removed only if enlarged (and mobile) or cytologically positive for malignant cells.

Unilateral or Bilateral Mastectomy. Glands 1 through 5 can be removed as a unit if multiple tumors or several large tumors preclude rapid, wide removal by lesser procedures. Simultaneous bilateral mastectomy also has been proposed and can be accomplished in dogs and cats with pendulous mammae, although staged, single chain mastectomies are better tolerated.[56] These procedures are done because they may be faster than multiple lumpectomies or mammectomies, not because they improve survival in the dog.

Lymph Node Removal. The axillary lymph nodes are rarely involved with mammary cancer in the dog

and should not be removed prophylactically. Fixed, adherent, and large axillary nodes can only rarely be removed completely. The inguinal lymph node should be removed when enlarged and cytologically positive for cancer or whenever gland 5 is removed, because it is intimately associated with this gland.

In summary, mammary cancer in the dog should be removed by the simplest procedure that will remove known cancer in the mammary gland. This does not mean that incomplete resection or debulking surgery is acceptable. In young, intact dogs with multiple lesions, the risk for future multiple operations would suggest consideration of more extensive surgery.

Chemotherapy

Although adjuvant chemotherapy often is the standard of care for breast cancer in humans, little information exists about its efficacy for mammary tumors in dogs. Based on established canine mammary tumor cell lines, doxorubicin has been shown to have antitumor activity using *in vitro* clonogenic assays; however, these assays rarely recapitulate *in vivo* correlates.[60] In one controlled study in a small number of dogs (n = 16), half received cyclophosphamide and 5-fluourouracil (5-FU) postoperatively, and half had regional mastectomy alone.[61] All dogs had stage III disease, and two in each group had histologically confirmed regional lymph node metastasis. When the chemotherapy-treated and control groups were compared by means of Kaplan-Meier analysis, statistically superior differences were seen in both the disease-free interval (24 months versus 2 months) and survival (24 months versus 6 months). Although the power of this study is low, it may support the use of adjuvant chemotherapy in certain cases.[61] Additional studies are needed to determine the optimal chemotherapeutic agent or agents for the treatment of canine malignant mammary tumors. Future clinical trials should concentrate on adjuvant chemotherapy of dogs with poor prognostic factors (e.g., large, lymph node–positive, invasive, high-grade tumors) after complete surgical removal.

Radiation therapy

As with chemotherapy, no reliable information on the value of radiation is yet available. Radiation therapy, like surgery, is mainly limited by the extent of the tumor and may only be considered useful in dogs with tumors that are too extensive for surgery. Patients with malignant tumors and "complete" resection do not need radiation. The role of radiation for malignant tumors with incomplete margins has not been defined in dogs. Radiation therapy (8 Gy in 2 or 3 fractions) has been tried in inoperable patients and patients with inflammatory carcinoma, resulting in anecdotal reports of palliation. More carefully designed studies are needed before the efficacy of irradiation for canine mammary tumors can be stated.

Biologic response modifiers

Studies of nonspecific immunomodulation using levamisole[56] and *Corynebacterium parvum* with bacillus Calmette-Guérin (BCG) combined with surgery show little effectiveness over surgery alone.[62] Based on an early study showing a favorable effect of intravenous BCG on survival, the WHO comparative oncology group performed a double-blind, prospective study on 130 bitches with confirmed mammary cancer treated by chain resection. The dogs were treated with intravenous BCG (live Pasteur strain), *C. parvum* vaccine, or intravenous (IV) saline. No statistical differences were reported in the 1-, 2-, and 3-year survival rates for the three treatment groups.[63] In another prospective study, no difference in recurrence rates was seen between dogs operated on for invasive cancer that received liposome muramyl-tripeptide phosphatidylethanolamine (L-MTP), a derivate from the mycobacterium cell wall that is encapsulated in liposomes, and dogs that received empty liposomes.[64] No validated, clinically applicable immunomodulating approach is available that can be considered to have proven therapeutic effectiveness.

Hormonal therapy

Although early ovariohysterectomy (OHE) clearly has a preventative role in the development of mammary tumors in dogs, the issue of OHE for therapeutic benefit remains unresolved. In one study of 154 dogs treated with concurrent mastectomy and OHE, the mean survival time was reported to be 8.4 months.[65] This offers no survival advantage compared to the mean survival times of 10 and 8 months in other studies for dogs treated by mastectomy alone.[44] More recent retrospective studies in mammary cancer did not show any benefit of OHE on the tumor-related[66] or overall death rate.[67] One study reported improved survival in dogs if the animal was ovariohysterectomized less than 2 years before or at the time of mammary cancer resection, compared to those that remained intact or were ovariohysterectomized more than 2 years before mammary cancer resection.[11] However, incomplete staging in this study and the presence of favorable prognostic factors (including tumor type) were more common in the first group of dogs. There have been *no well-designed prospective studies* that clearly address the issue of OHE as an adjunct treatment for dogs with malignant mammary tumors.

Antiestrogens, such as tamoxifen, have been tested both *in vitro* for antiproliferative activity in canine mammary tumor cell lines[60] and to a limited degree in clinical cases.[68,69] Postulated adjuvant antitumor activity by tamoxifen could not be documented in a study of limited size in 18 dogs after mastectomy and OHE because of frequent (10 of 18 dogs) estrogen-like signs related to tamoxifen side effects. These side effects include vulvar swelling, vaginal discharge, incontinence,

Box 26-3	Summary of Canine Mammary Tumor Prognostic Factors*	

Good	Poor	Indifferent
<3 cm in diameter	>3 cm in diameter	Age
Well circumscribed	Invasive, ulcerated	Breed (small breeds may have more benign tumors[8])
Lymph node: Negative	Lymph node: Positive	OHE status at time of surgery
ERs or PRs: Positive	ERs: Negative	Weight
Histologic subtype (carcinoma—well differentiated, complex, tubular/papillary)	Histologic subtype (carcinoma—poorly differentiated, simple, solid, anaplastic; inflammatory carcinoma; sarcomas)	Type of surgery (simple or radical)
Tumor grade I	Tumor grade III	Number of tumors

Index of proliferation
  AgNOR: Low count
  Ki-67: Low
  PCNA: Low
	Index of proliferation
	  AgNOR: High count
	  Ki-67: High
	  PCNA: High
	  p53 gene mutation
	  DNA aneuploidy
		Glands involved

*These factors should serve as relative indicators of the prognosis; individual variation occurs.

OHE, Ovariohysterectomy; ERs, estrogen receptors; PRs, progesterone receptors; AgNOR, argyrophilic nucleolar organizer region, PCNA, proliferating cell nuclear antigen.

urinary tract infection, stump pyometra, and signs of estrus.[69] At this time, tamoxifen therapy is not advised in dogs.

Prognosis

Based on both univariate and multivariate studies, the following factors have been determined to be prognostic: tumor size, lymph node involvement, presence of distant metastasis, histologic type, malignancy grade, degree of nuclear differentiation, evidence of lymphoid cellular reactivity in the tumor vicinity, degree of invasion, intravascular growth, steroid hormone receptor activity, S-phase fraction as a measure of proliferation, deoxyribonucleic acid (DNA) aneuploidy, and number of silver-staining nucleolar organizer regions (AgNORs) (Box 26-3).* Factors that do not seem to be associated with the prognosis are the tumor location, number of tumors present, type of surgery (as long as histologically adequate resection is achieved), and OHE at surgery, although controversy exists regarding the importance of age at diagnosis.[9,57,70-74] The influence of breed according to the patient's size or weight also has been evaluated. One study found that small breed was associated with longer survival, but also that small breed dogs had

a higher incidence of benign tumors.[8] In dogs with malignant tumors, breed and weight have not been shown to influence survival.[74]

One study found a correlation between the histologic grade (as described previously) and the disease-free interval after mastectomy in 158 dogs with mammary cancer.[45] Only 19% of the dogs with grade 0 (*in situ* or noninvasive) carcinomas had recurrence or metastasis within 2 years after initial mastectomy compared to 60% of dogs with grade I disease (stromal invasion) and 97% of dogs with grade II disease (invasion into vascular or lymphatic vessels). The prognosis is very good for dogs with noninvasive carcinoma (Figure 26-3).

The degree of nuclear differentiation (i.e., poorly, moderately, or well-differentiated tumors) also has been shown to be an important factor in the prognosis. The risk of developing recurrent or metastatic carcinoma in less than 2 years after mastectomy was 90% for dogs with poorly differentiated tumors, 68% for those with moderately differentiated tumors, and only 24% for those with well-differentiated carcinoma.[45] A report using a different grading scheme also showed prognostic significance for nuclear differentiation; patients with grade III tumors had a shorter survival time and an greater likelihood of tumor-related death.[49] In this study, tumor size did not influence grade and was not associated with survival, but lymph node metastasis worsened the prognosis.

Figure 26-3

Kaplan-Meier disease-free interval curve comparing histologic grade after mastectomy in 158 dogs with malignant mammary tumors. Grade 0, n = 35; grade I, n = 76; grade II, n = 47. The three stages are significantly different; p <0.0001. *(From Kurzman ID, Gilbertson SR: Semin Vet Med Surg 1:25-32, 1986.)*

Figure 26-4

Kaplan-Meier disease-free interval curve comparing tumor size after mastectomy in 54 dogs with locally invasive, malignant mammary tumors. Dogs with tumors smaller than 3 cm in diameter (n = 16) had significantly longer disease-free intervals than dogs with tumors 3 to 5 cm (n = 20) or larger than 5 cm (n = 18); p <0.04. *(From Kurzman ID, Gilbertson SR: Semin Vet Med Surg 1:25-32, 1986.)*

Lymphoid cellular reactions in the tumor vicinity are another factor that has been correlated with the prognosis. Lymphoid cellular activity may indicate morphologic evidence of an antitumor immune response. In one study, dogs with mammary cancer that did not have evidence of lymphoid cellular activity at the time of initial mastectomy had a significantly higher risk of developing recurrence within 2 years than those with reactivity.[45] In this study, dogs with histologic grade I tumors showing lymphoid cellular reactivity had a 45% recurrence rate within 2 years, and dogs without cellular reactivity had an 83% recurrence rate within 2 years.

Mammary sarcomas are considered to have a poor prognosis.[72,75] Most dogs with sarcomas die of the disease within 9 to 12 months. With carcinomas, the histologic subtype has been shown to influence survival. In one study of 99 dogs, those with anaplastic carcinoma had a worse postoperative survival in univariate analysis (median survival, 2.5 months) than dogs with adenocarcinoma (median survival, 21 months), solid carcinoma (median survival, 16 months), and other types of tumors (median survival, 24 months).[74] However, this finding did not hold up in multivariate analysis. In another report, the tumor grade was associated with the histologic subtype, with simple carcinomas having a higher percentage of grade III undifferentiated tumors (50%) than grade I tumors (18%). Inflammatory carcinomas also have a poor prognosis.[51,54] Most cannot be resected surgically, and if resected, they tend to recur within weeks to 1 month after surgery. These patients also often have some degree of disseminated intravascular coagulation, and excessive bleeding frequently occurs at the time of surgery. In one study, 33 dogs with IC had a mean survival time of 25 days with palliative care.[50]

Tumor size is an important prognostic factor (Figure 26-4). The WHO clinical staging system categorizes dogs according to the diameter of the largest malignant tumor (see Box 26-2). In dogs with locally invasive disease, significant differences have been found between T_1 and T_2, and T_1 and T_3 tumors, and in some but not other studies between T_2 and T_3 tumors.[67,70,74] Dogs with invasive cancer that have tumors smaller than 3 cm in diameter have a significantly better prognosis than dogs with malignant tumors 3 cm in diameter or larger (Figure 26-4). In one study of a group of dogs with invasion of lymphatic vessels or lymph node metastasis, no significant differences were found among T_1, T_2, and T_3 tumors.[70] In another study, dogs with a

T_3 carcinoma (over 5 cm in diameter) had a median survival time of 40 weeks, compared to 112 weeks for animals with smaller tumors.[76] In yet another study, dogs with tumors smaller than 3 cm in diameter survived significantly longer (22 months) than dogs with tumors larger than 3 cm (14 months).[74]

In univariate analyses, lymph node involvement predicted poorer disease-free survival, with 80% of the dogs with lymph node involvement having recurrence within 6 months.[45,70,73] In contrast, dogs with mammary carcinoma (excluding sarcomas) with negative lymph nodes usually have a recurrence rate of 30% or lower by 2 years after surgery[70] (Figure 26-5). Karayannopoulou and colleagues[49] also reported that lymph node metastasis had a negative impact on survival. During a 2-year follow-up period, 24 of 28 patients (86%) with positive lymph nodes died of disease, compared to 8 of 38 patients (21%) that did not have lymph node metastasis.

The presence of metastatic disease at the time of diagnosis also has a worse prognosis. In one study, the median postoperative survival was 5 months, compared to 28 months for dogs without metastasis at presentation.[74] In another study, the presence of ERs or PRs (or both) above a certain threshold (10 fmol/mg cytosolic protein) correlated with improved survival in

45 dogs with mammary cancer after surgery.[13] Whether the presence of steroid receptors is an independent prognostic factor is questionable, because the presence of these receptors has been correlated with well-differentiated tumors, and they have been found more often in complex than simple carcinomas.[77] A study involving 84 patients looked at receptor status as a prognostic indicator for disease-free progression (DFP). Although univariate analysis indicated that ER+ or PR+ or both had a favorable influence on DFP, this did not hold up in multivariate analysis.[18]

In a study of 136 dogs with mammary cancer and adequate follow-up, both the presence of DNA aneuploidy and a high S-phase percentage (a measure of the proliferation rate) were associated with reduced survival time.[72] Multivariate analyses diminished the value of DNA ploidy status, leaving a high S-phase fraction (SPF), sarcoma, and age as important prognostic factors that increased the hazard of death from tumor or unknown cause.[72] Other immunohistochemical techniques detect the presence of antigens expressed during the cell cycle, including Ki-67 and proliferating cell nuclear antigen (PCNA). Of these, the Ki-67 labeling index recently was indicated to be of prognostic value.[78]

Additional investigation has been done using cytologic preparations instead of routine histologic samples. In a study of 31 dogs, the Ki-67 proliferation indices were lower for nonmalignant tumors, and high Ki-67 index values correlated with metastasis, death from disease, and a low overall disease-free interval (DFI) and survival rate.[79] In addition, a high AgNOR count (another proliferation marker) was implicated as a prognosticator in canine mammary cancer.[80] In one study, multivariate analysis indicated that a mutation in the p53 gene conferred an increased risk of tumor recurrence and death from mammary tumor.[31] Continued investigation into these and other molecular markers hopefully will lead to clinically useful predictors of the prognosis or, more important, will define potential targets for therapeutic strategies.

FELINE MAMMARY TUMORS

Mammary tumors are the third most common tumor in the cat, after hematopoietic neoplasms and skin tumors.[1,81-83] The incidence of mammary tumors in the cat is less than half that seen in humans and dogs; however, these tumors account for 17% of neoplasms in female cats.[1,82-84] Mammary tumors also have been reported in male cats, although less often (1% to 5% of feline mammary tumors).[85,86] In contrast to humans and dogs, at least 85% of feline mammary tumors are malignant.[76,81-83]

Some evidence indicates a breed-associated predilection for mammary tumors; domestic shorthair and

Figure 26-5

Kaplan-Meier disease-free interval curve comparing lymph node metastasis after mastectomy in 45 dogs with malignant mammary tumors. Dogs in which the lymph nodes tested negative (n = 26) had a significantly longer disease-free interval than those in which the lymph nodes tested positive (n = 19); p <0.001. *(From Kurzman ID, Gilbertson SR: Semin Vet Med Surg 1:25-32, 1986.)*

Siamese cats appear to have higher incidence rates.[82,87,88] Siamese cats may have twice the risk of any other breed of developing mammary tumors.[86] Mammary neoplasia has been reported to occur in cats from 9 months to 23 years of age (mean age, 10 to 12 years).[82-90] One study suggests that the disease occurs at an earlier age in Siamese cats and that the incidence in this breed reaches a plateau at 9 years of age.[82] The mean age reported for a group of 39 male cats (12.5 years) was slightly higher than that seen in females.[85]

Hormonal influences probably are involved in the pathogenesis of mammary tumors in the cat. Dorn and coworkers[1] found that cats ovariectomized at 6 months of age had approximately a sevenfold reduced risk of mammary cancer than intact cats. More recently, in a case control study by Overly and colleagues,[91] cats spayed before 6 months of age had a 91% reduction in their risk of mammary carcinoma compared to intact cats, and cats spayed before 1 year of age had an 86% reduction in risk. A strong association also has been documented between previous use of drugs containing synthetic progestins or estrogen-progestin combinations and the development of benign or malignant mammary tumors in cats; in both cases, the risk was more than threefold over that in untreated cats.[92] A benign fibroepithelial hyperplasia may be seen in some cats recently exposed to sex steroids.

Evidence that sex steroids may interact with mammary cells and thereby influence mammary tumorigenesis comes from the observation that both normal tissue and benign proliferative lesions frequently express low levels of ERs and moderate levels of PRs.[13,93,94] A less frequent expression of ERs and PRs in feline mammary carcinomas suggests loss of steroid dependence during malignant progression.[13,93,95] Because of this and other similarities (e.g., histopathologic appearance and pattern of metastasis), feline mammary carcinoma has been proposed as a useful comparative model for hormone-independent human breast carcinomas.[96] To this end, recent molecular studies have evaluated HER2/neu overexpression in feline mammary carcinomas. When amplified and overexpressed in human breast cancer, HER2/neu (c-erbB-2) has been associated with clinically aggressive tumors and an overall poorer prognosis. This overexpression is reported to occur in 10% to 40 % of human breast carcinomas.[97,98] Millanta and colleagues[97] reported the same overexpression in 59% of 47 cases of feline mammary carcinoma studied and found it to be significantly associated with a shorter overall survival. A second study reported that the feline HER2 gene kinase domain had 92% homology to its human counterpart; it also found HER2 overexpression in 39% of mammary carcinomas tested.[98]

Other molecular targets have been investigated in feline mammary tumors to elucidate the prognosis or the pathways of tumorigenesis. Many of these targets suggest an analogy between feline mammary carcinoma and aggressive human breast cancer. In one study, cyclin A, which acts as a regulatory protein in cell cycle progression, was found in 46% of tumors tested but was absent in benign mammary tissue.[99] Immunohistochemical staining for vascular endothelial growth factor (VEGF) also has been investigated in malignant tissues in cats, and a higher percentage of cells that stained positive for VEGF has been correlated with an unfavorable prognosis.[100] E-cadherin, a cell adhesion molecule, has been shown to be reduced or absent in 70% of feline mammary carcinomas compared to normal tissue.[101] Reports on the importance of p53 overexpression or mutations are conflicting with respect to feline mammary carcinomas.[102,103]

Pathology and Natural Behavior

Mammary tumors and dysplasias

Between 85% and 93% of feline mammary tumors are histologically malignant. Many of the tumors, especially the large, more invasive neoplasms, adhere to the skin and are ulcerated. Lymphatic and lymph node invasion is common.[84,87,90] In several studies, more than 80% of cats with mammary malignancy had metastases to one or more of the following organs at the time of death: lymph nodes, lungs, pleura, liver, diaphragm, adrenal glands, and kidneys.[82,84,87,89,104]

More than 80% of feline mammary tumors are classified histologically as adenocarcinomas.[83-87] The frequency of diagnosis of the specific types of adenocarcinomas differs slightly among pathologists, but most agree that tubular, papillary, solid, and cribriform carcinomas are the most common, and some carcinomas show a combination of histologic types in one lesion. Sarcomas, squamous cell carcinomas, and mucinous carcinomas are less common malignancies. Inflammatory mammary carcinoma recently was reported in three cats based on the rapidly progressing clinical signs and on the associated inflammation and dermal lymphatic invasion.[105]

Approximately 15% of mammary masses are benign neoplasms or dysplasias, including simple/complex adenomas and fibroadenomas.[81,84,87,89,104] In addition, there are three types of noninflammatory hyperplasia of the feline mammary gland: ductular hyperplasia, lobular hyperplasia, and fibroepithelial hyperplasia.

Fibroepithelial hyperplasia

Fibroepithelial hyperplasia[81] may occur in young cats shortly after a (silent) estrus from about 6 months of age, or during pregnancy until 2 years of age. This has also been reported in both male and female cats treated with exogenous progestins.[94,106-110] One or (more often) several glands may be enlarged, and sometimes massive, two-sided enlargement is seen (Figure 26-6).

Figure 26-6

Gross appearance of fibroepithelial hyperplasia in a young cat.

This is thought to result from hormonal stimulation of the glandular tissue. The mass may become erythematous, ulcerated, and necrotic, leading to bleeding and localized infection. Edema of the skin and subcutis is common and occasionally may extend to the rear legs. Fibroepithelial hyperplasia can also resemble acute mastitis. Treatment involves removal of the hormonal influence, if possible; regression has been seen after termination of pregnancy in some affected animals. Ovariohysterectomy was found effective in most cases, with gradual regression of the masses over weeks to months. If an OHE is to be performed and the glands are still greatly enlarged, a flank incision should be considered. In animals experiencing the influence of long-acting, injectable progestins, removal of the hormonal influence may be difficult, and clinical signs may not resolve with OHE.[109]

History and Signs

Feline mammary cancers often are in an advanced state of development by the time the cat is brought to the veterinarian.[88] The neoplasm may adhere to the overlying skin or the underlying abdominal wall. The tumor usually is firm and nodular. At least 25% of affected patients have ulcerated masses. The involved nipples may be red and swollen and may exude a tan or yellow fluid. The tumor may involve any or all of the mammary glands without left-right or cranial/caudal predeliction.[87,88,90,104] More than half of affected cats have multiple gland involvement.[84,85,87,88] Metastatic lung and thorax involvement may be extensive and may cause respiratory insufficiency as a result of a pleural carcinomatosis with an effusion, often containing malignant cells.

Diagnostic Techniques and Workup

Before any diagnostic or therapeutic steps are taken, the cat's health status must be fully assessed. A serum chemical profile, urinalysis, and complete blood count should be done to identify any presurgical abnormalities. The number, site, and size of primary tumors should be recorded, together with possible signs of fixation. Alterations in the size and consistency of lymph nodes should be assessed, and any suspected node should be aspirated or biopsied. Thoracic radiographs should be taken in both the right and left lateral and ventrodorsal planes to search for pulmonary, lymph node, and pleural metastases. Mammary tumor pulmonary metastases appear as interstitial densities. They range from those that are faintly seen, to those that are several centimeters in diameter, to miliary pleural lesions than can produce significant effusion. Sternal lymphadenopathy occasionally is seen.

Because these tumors so often are malignant, an aggressive approach should be taken to confirm the diagnosis. Except for cases in which fibroepithelial hyperplasia is suspected based on the history and clinical signs, a preliminary biopsy usually is not recommended, because 80% to 85% of the masses in a mammary gland will be malignant. However, cytology may be helpful for ruling out nonmammary malignancies. Tissue for histopathology is taken at the time of mastectomy.

Clinical Staging

The most important goals of staging of malignant tumors (Box 26-4) are to (1) evaluate the primary tumor or tumors, (2) evaluate the regional lymph nodes, and (3) identify any distant metastatic sites.

The size of the primary tumor and clinical evidence of invasiveness (fixation to skin or fascia) lead to the proper *T* category in the tumor-node-metastasis (TNM) system. The regional lymph nodes should be examined carefully, and fine-needle aspiration or surgical removal may be necessary to determine metastasis and to help categorize the *N* status. In the *M* category, any sign of distant metastasis establishes M_1 status; this includes involved distant lymph nodes, pleural effusion proven to contain tumor cells or radiographic signs of metastatic disease in the lungs, or radiographic or ultrasonographic signs of lymph node involvement in the thorax or abdomen.

Surgery

Mammary neoplasms in the cat have been treated in a variety of ways. Surgery is the most widely used treatment. It may be used alone or in combination with chemotherapy or other modes of cancer therapy.

The success of surgery is hindered by the invasive nature of the disease and its tendency for early metastasis. Chain mastectomy (i.e., removal of all four glands on the affected side) is the surgical method of choice because it significantly reduces the chance of local tumor recurrence.[88,110-112] This procedure frequently is used regardless of the size of the tumor. The cat, unlike the dog, usually has four pairs of mammary glands. The cranial two glands on each side have a common lymphatic system and drain into the axillary lymph nodes and then to sternal nodes. The caudal two glands drain to inguinal lymph nodes. In contrast to the dog, in which more conservative resections may be appropriate in carefully selected cases, most cats require a complete unilateral or bilateral mastectomy. Tumor fixation to skin or abdominal fascia necessitates en bloc removal of these structures. Complete unilateral mastectomy usually is performed if the tumor (or tumors) is confined to one side. Staged mastectomy (2 weeks apart) or simultaneous bilateral mastectomy is done when tumors are bilateral. The inguinal lymph node is almost always removed with gland 4, whereas the axillary lymph nodes are removed only if they are enlarged and cytologically test positive for tumor. Aggressive or prophylactic removal of axillary nodes is unlikely to have therapeutic benefit.[88,110-112]

Although OHE has not been shown to reduce the incidence of recurrence, some believe it is warranted.[82,88,113] If the mammary mass was caused by a benign condition (e.g., fibroepithelial hyperplasia), OHE often results in regression of the hyperplastic tissue (see earlier discussion).

Radiation therapy

Radiation therapy is not used routinely to treat feline mammary tumors. Currently, no evidence indicates that radiation improves the survival rate of patients with feline mammary tumors.

Chemotherapy

Chemotherapy with doxorubicin (25 mg/m^2 given intravenously slowly) every 3 weeks alone or with cyclophosphamide (50 to 100 mg/m^2 given orally on days 3, 4, 5, and 6 after doxorubicin) has induced a short-term response in about half of cats with metastatic or nonresectable local disease.[114-116] Partial responses (more than 50% regression) were noted with doxorubicin alone (nine of 14 cats) and with doxorubicin with cyclophosphamide (seven of 14 cats). The chemotherapy protocol can be repeated every 3 to 4 weeks. A recent retrospective study that evaluated single agent, adjuvant doxorubicin therapy (1 mg/kg given intravenously every 3 weeks for five total treatments) after surgical excision in 67 cats reported a Kaplan-Meier median survival time of 448 days, with 59% of the patients alive at 1 year, 37% at 2 years, and 17% at 5 years.[117] The median DFI for the same study population was 255 days. This study suggests that the use of chemotherapy as an adjuvant holds promise for improved outcome, but controlled, randomized trials are needed to prove its true efficacy. In addition, doxorubicin can be nephrotoxic in cats,[118] and careful evaluation of renal function is recommended.

Biologic response modifiers

Studies using nonspecific biologic response therapy, such as levamisole[111] and bacterial vaccines,[112] have
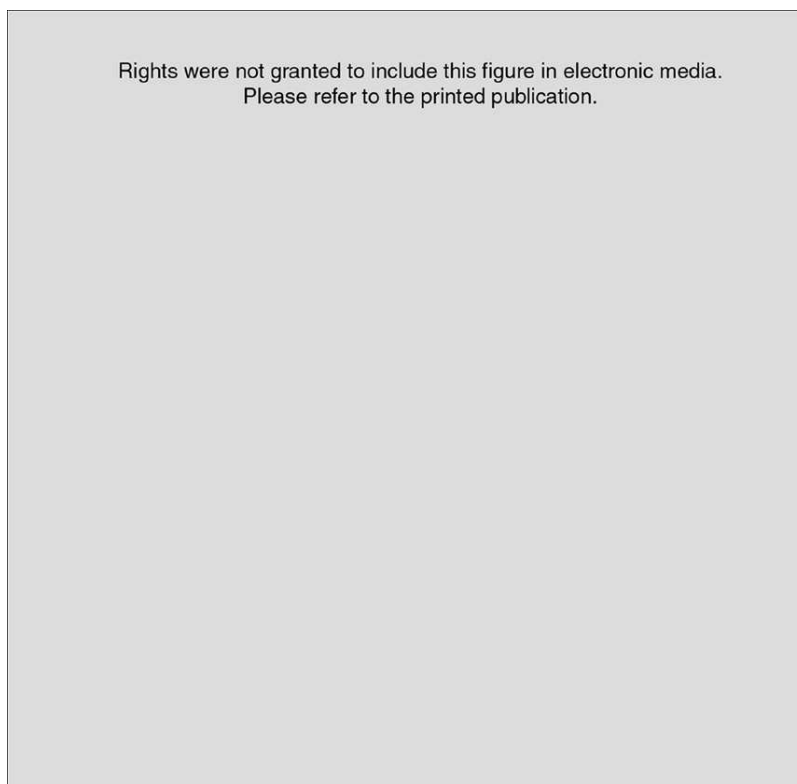
Figure 26-7

Kaplan-Meier survival curve comparing tumor volume after mastectomy in cats with malignant mammary adenocarcinoma. Statistically, tumor volume is highly significant as a prognostic factor. *(From MacEwen EG, Hayes AA, Harvey HJ et al: J Am Vet Assoc 185:201-204, 1984.)*

shown minimal effect on reducing the recurrence or prolonging the survival time in cats when combined with surgery. The use of L-MTP after mastectomy has not shown any significant reduction in local recurrence or survival compared to surgery alone.[119] To date, no biologic response modifier has proved efficacious in cats with mammary cancer.

Prognosis

Little progress has been made over the past 20 years in extending the survival time of patients with feline mammary tumors. Because stromal invasion almost always is present and metastases frequently are present at the time of surgery, a guarded to poor prognosis should always be given. With conservative surgery, two thirds of cats in which the tumors are surgically excised have a recurrence at the surgical site.[88,110,112] Most studies state that the interval from tumor detection to the death of the cat is 10 to 12 months.[82,83,88,104,110]

The most significant prognostic factors with regard to recurrence and survival with feline malignant mammary tumors are the *tumor size*,[104,110,112] (Figure 26-7), *extent of surgery*[110] (Figure 26-8), and *histologic grade*.[104,117,120-122] Cats with tumors larger than 3 cm in diameter reportedly have a median survival time of 4 to 12 months.[120,122] Cats with tumors 2 to 3 cm in diameter have a significantly better median survival time of 15 to 24 months, and cats with tumors smaller than 2 cm in diameter have a median survival time of longer than 3 years. Therefore both the owner and the practitioner should realize that early diagnosis and aggressive treatment are important prognostic factors with malignant feline mammary tumors.

Few studies have reported on the significance of lymph node metastasis with regard to the prognosis; however, those that do validate the intuitive negative effect of nodal metastasis.[122,123] A recent multiinstitutional trial in cats treated with surgery followed by doxorubicin found that in cats that developed metastatic disease, the location of the metastasis had prognostic significance.[117] In this study, the survival time for patients with pulmonary metastasis was 331 days, and for those with nodal metastasis, it was longer than 1500 days,

Figure 26-8

Kaplan-Meier disease-free interval curve comparing the results of conservative surgery to those of radical mastectomy in cats with malignant mammary adenocarcinoma. Cats that undergo radical mastectomy have a statistically significant reduced rate of local recurrence. *(From MacEwen EG, Hayes AA, Harvey HJ et al: J Am Vet Assoc 185:201-204, 1984.)*

although the number of patients that developed nodal metastasis was small.

Very few studies have been performed to evaluate the effectiveness of the extent of local therapy in malignant feline mammary tumors. One study showed that radical mastectomy reduced local recurrence but did not increase the overall survival time (see Figure 26-7).[112] More recently, Novosad and colleagues[117] found that the extent of surgery had a significant effect on survival in a subset of cats treated at a single institution; those that underwent bilateral radical mastectomies survived for 917 days, those that had regional mastectomies survived for 428 days, and those that had unilateral mastectomies survived for 348 days.

Another prognostic factor with malignant mammary tumors is the degree of nuclear differentiation. Well-differentiated tumors with few mitotic figures (indicative of a low proliferation rate) have correlated with an improved survival time.[104] Unfortunately, this tumor type is rare compared to the more undifferentiated forms. In addition, a high proliferation index, as assessed by measurement of Ki-67–positive cells, appeared to be related to reduced survival in cats that underwent surgery for mammary carcinoma.[123] Other phenotypic factors that may be indicative of the prognosis include VEGF expression and HER2/neu overexpression.[97,100]

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27

Tumors of the Male Reproductive System

Timothy M. Fan and Louis-Philippe de Lorimier

Malignantly transformed cells of the testes, prostate gland, penis, and associated structures account for most tumors of the male reproductive system encountered in dogs and cats. Testicular tumors generally are biologically nonaggressive in dogs, and most can be cured with simple orchiectomy. In rare cases functional or metastatic testicular tumors can cause significant morbidity or mortality. Several factors may influence the development of testicular tumors, including age, breed, cryptorchidism, and exposure to environmental carcinogens. In contrast to testicular tumors, the clinical behavior of canine prostatic carcinoma is biologically aggressive, as demonstrated by early regional and distant metastases. The etiology of canine prostatic carcinoma remains poorly defined. The influence of androgens in canine prostatic carcinogenesis has been recently investigated, and it appears that most canine prostatic tumors may not be androgen dependent. Tumors of the penis, os penis, and prepuce may be epithelial, mesenchymal, or hematopoietic in origin. Tumors of the feline male reproductive system are rare, but testicular and prostatic malignancies have been reported.

CANINE TESTICULAR TUMORS

Incidence

In the intact male dog, the testis is the second most common anatomic site for cancer development, and testicular tumors account for approximately 90% of all cancers arising from the male genitalia.[1-3] Primary testicular tumors may be derived from three specialized testicular elements: the interstitial cells of Leydig, the sustentacular cells of Sertoli, and the spermatic germinal epithelium.[2] Subsequent to malignant transformation, these testicular elements respectively give rise to interstitial cell tumors, Sertoli cell tumors, and seminomas. These three tumor types occur with approximately equal frequency and as a group account for most diagnosed testicular tumors in dogs.[3] In rare cases testicular cancers may develop from other cell lineages, giving rise to various tumor types, including hemangiomas, granulosa cell tumors, teratomas, sarcomas, embryonal carcinomas, gonadoblastomas, lymphomas, and rete testis mucinous adenocarcinomas.[4-6] *De novo* formation of multiple testicular tumors in the same patient is common, and approximately 40% of dogs diagnosed with testicular cancer have more than one primary tumor.[1,7,8] In the descended testes, no predilection for cancer development in either the right or the left testis has been recognized. However, cryptorchid testes have a higher incidence of Sertoli cell tumors and seminomas, with the right testis more often being retained and therefore predisposed to tumorigenesis.[9-11] Testicular tumors are most often diagnosed in geriatric male dogs (median age, about 10 years).[1,12]

Risk Factors

Several factors have been shown to increase the risk of primary testicular tumors. A recognized risk factor that favors the development of Sertoli cell tumors and seminomas is the local tissue environment associated with intra-abdominal or inguinal testes retention. An epidemiologic study compared the incidence of testicular tumors in cryptorchid and normal, age-matched control dogs.[11] None of the control dogs developed testicular tumors for the duration of the study, whereas cryptorchid dogs older than 6 years had a calculated incidence of testicular neoplasia of 68.1 per 1000 dog-years at risk. Another study demonstrated that 54% of Sertoli cell tumors and 34% of seminomas diagnosed in intact male dogs developed in cryptorchid testes.[13] Although cryptorchidism is a definitive risk factor for primary testicular tumorigenesis, other factors, including age, breed, and exposure to environmental carcinogens, may positively influence the development of tumors. In one study that evaluated cryptorchid dogs, chronologic age was determined to be a risk factor for the formation of primary testicular tumors.[11] In this study, dogs older than 10 years were more likely to develop tumors than younger dogs (i.e., those under 6 years of age).

Certain breeds appear to have an increased risk of developing primary testicular tumors, including the boxer, German shepherd, Afghan hound, weimaraner, and Shetland sheepdog.[10,12-14]

Exposure to environmental carcinogens has been associated with an increased incidence of seminomas in military working dogs that served in the Vietnam War. In two epidemiologic studies, necropsies conducted on deceased military service dogs revealed pathologic testicular changes, such as testicular hemorrhage, epididymitis, orchitis, sperm granuloma, testicular degeneration, and seminomas.[16,17] The causative factor or factors for the development of seminomas in military working dogs has not been definitively determined. However, it was speculated that exposure to phenoxy herbicides, dioxin, or tetracycline during the dogs' Vietnam duty may have promoted the development of testicular tumors.[16,17]

Pathology and Natural Behavior

In the dog, most primary testicular tumors remain locally confined. Regional or distant metastasis is uncommon, occurring in fewer than 15% of dogs diagnosed with either Sertoli cell tumors or seminomas and even less frequently in those with interstitial cell tumors. When testicular tumors behave aggressively, metastatic lesions initially may involve the regional draining lymph nodes but ultimately may spread to distant sites, including the liver, pulmonary parenchyma, kidneys, spleen, adrenals, pancreas, skin, eyes, and central nervous system.[8,10,18-22]

In recent years, investigators have attempted to elucidate the molecular and cellular biology of primary testicular tumors in dogs. The cell proliferative activities of seminomas, Sertoli cell tumors, and interstitial cell tumors have been characterized with immunohistochemical staining for proliferating cell nuclear antigen (PCNA) and Ki-67. Although no quantitative differences in either PCNA or Ki-67 were identified among the three primary tumor types, an increase in proliferative indices in Sertoli cell tumors and seminomas was associated with morphologic progression from tubular to diffuse patterns.[23] These results suggest that as testicular tumors become more anaplastic, they may be provided with a proliferative advantage compared to well-differentiated tumors. In another study, differences in cell proliferation in 20 seminomas of varying morphologic subtypes and tissue invasiveness were assessed by silver-stained nucleolar organizing regions (AgNORs).[24] The mean AgNOR counts per cell were higher in invasive intraductal or diffuse seminomas than in confined, well-differentiated intraductal seminomas; however, AgNOR counts should not be used prognostically for testicular tumors until similar results are corroborated by larger studies with greater statistical power.

Related to cell proliferative indices, the expression of cyclins in primary testicular tumors has been investigated. Cyclins combine with cyclin-dependent kinases (CDKs) to form protein complexes that regulate cell cycle checkpoints. Cyclins D1 and E have been recognized as proto-oncogenes and are overexpressed in several human cancers. In one study, the expression of cyclins A, D1, D2, and E in primary testicular tumors was compared with that in normal canine testes.[25] Cyclin A was identified in all normal testes and in most seminomas but was not detected in Sertoli cell and interstitial cell tumors. Cyclins D1 and E were not identified in any primary testicular tumors, which suggests that canine testicular tumorigenesis may not stem from alterations in critical cell cycle regulators.[25]

Primary testicular neoplasms, such as Sertoli cell tumors, can cause signs of feminization. To better understand the role of sex hormones in the pathology of testicular tumors, researchers have evaluated the blood plasma concentrations of these hormones in dogs with primary testicular tumors. In one study, 20 dogs with interstitial cell tumors, six dogs with Sertoli cell tumors, and nine dogs with seminomas were evaluated for levels of circulating estradiol-17β and testosterone, which were compared with levels in healthy control dogs.[26] Estradiol concentrations were considerably higher in dogs with Sertoli cell tumors and lower in dogs with seminomas compared with healthy controls. In addition, testosterone concentrations in dogs with Sertoli cell tumors were lower than in healthy dogs. When testosterone/estradiol ratios were compared, dogs with Sertoli cell tumors had significantly lower ratios than the healthy controls. Clinical signs of feminization secondary to Sertoli cell tumors correlated best with reductions in the testosterone/estradiol ratio rather than absolute increases in estradiol-17β; this suggests that the relative ratio of sex hormones may predict which testicular tumors are more likely to induce clinical signs of feminization.

In addition to testosterone and estradiol levels, expression of other hormones, such as inhibin isotypes (α, βB, and βA) and 3β-hydroxysteroid dehydrogenases (3β-HSDs) recently have been investigated in primary canine testicular tumors. Inhibin hormones selectively prevent the secretion of follicle-stimulating hormone from the pituitary gland; 3β-HSD is a steroidogenic enzyme found in Leydig's cells. Interstitial cell tumors, but not Sertoli cell tumors or seminomas, produce inhibins and 3β-HSD, allowing for discrimination of interstitial cell tumors from other tumors of the canine testes.[27]

Insulin-like growth factor-I (IGF-I) and IGF-II are peptides involved in cellular growth and differentiation. Through interactions with their cognate receptors and specific binding proteins, IGF peptides can exert both

autocrine and paracrine cell proliferative effects. The IGF system plays an important role in local regulation of testicular function, and derangements in this pathway may contribute to testicular malignant transformation. Furthermore, because steroid hormone metabolism and transport are responsible for hormone-mediated cell proliferation in the testes, dysregulated expression of steroid hormone metabolic genes may promote testicular tumorigenesis. Recently, the expression of genes coding for IGF-I, IGF-II, IGF-I receptor, and five associated binding proteins (IGFBP1-5), as well as genes for androgen receptor, p450-aromatase enzyme, and 5α-reductase type I and II were evaluated in normal testes and primary testicular tumors.[28] Relatively small differences in IGF-related gene expression were observed between normal testes and testicular tumors, which suggests that the IGF system may not be important for canine testicular tumor maintenance and growth. Differences in steroid hormone metabolic genes were identified between normal testes and primary testicular tumors. Sertoli cell tumors and seminomas showed lower expression of 5α-reductase type I than normal testes, which indicates a reduced capacity to form androgens in these primary testicular tumors.[28]

Angiogenesis plays an important role in cancer progression. Surrogate markers of the angiogenic process, specifically vascular endothelial growth factor (VEGF) and microvessel density, have been evaluated by means of quantitative immunohistochemical analysis in normal canine testes and seminomas of differing histologic subtype (intratubular versus diffuse).[29] Both VEGF expression and microvessel density were higher in seminomas than in normal testicular tissues. In addition, anaplastic diffuse seminomas had greater VEGF expression and microvessel density than well-differentiated, intratubular seminomas.[29] Similar to other tumors, these findings suggest that progression of canine seminomas may be partly mediated through the enhancement of angiogenic processes.

Figure 27-1

Large Sertoli cell tumor with atrophy of the contralateral testis. *(Photograph courtesy Dr. Sandy Manfra.)*

Figure 27-2

Intra-abdominal cryptorchid testis with secondary development of a Sertoli cell tumor (shown during surgical excision). *(Photograph courtesy Dr. Sandy Manfra.)*

History and Clinical Signs

Clinical signs associated with canine testicular tumors are variable and can be caused by the physical presence of the primary tumor itself or by metastases, or they may be a consequence of paraneoplastic syndromes. Testicular tumors usually are diagnosed by palpation of an enlarged testicle or a testicular mass during a routine physical examination, ultrasonographic studies of the scrotum and abdomen, or necropsy. Atrophy of the contralateral normal testicle is common (Figure 27-1). In cryptorchid dogs, testicular tumors may result in regional mass effects in the abdominal cavity or inguinal space (Figure 27-2). Hyperestrogenism, a paraneoplastic manifestation of primary testicular

tumors, may cause signs of feminization, including bilaterally symmetric alopecia and hyperpigmentation, a pendulous prepuce, gynecomastia, galactorrhea, atrophic penis, and squamous metaplasia of the prostate (Figure 27-3).[10] Sertoli cell tumors arising from intra-abdominal cryptorchid testes are most commonly associated with hyperestrogenism, although both seminomas and interstitial cell tumors can cause hormonal imbalances.[30-35] In addition to cutaneous and genital changes, hyperestrogenism may have life-threatening and irreversible hematologic consequences. Blood dyscrasias and bone marrow hypoplasia, manifesting as pancytopenia, often is fatal (Figure 27-4).[30] Less common

clinical signs associated with primary testicular tumors are hematuria, spermatic cord torsion, and hemoperitoneum.[36]

Diagnostic Techniques and Workup

Although identification of a testicular mass is supportive, histopathologic evaluation is required for definitive diagnosis of a testicular neoplasm. For valuable breeding animals, fine-needle aspiration with cytology of the testicular lesion may provide a presumptive diagnosis before irreversible orchiectomy. Although most primary testicular tumors remain locally confined, affected dogs

Figure 27-3

Gynecomastia and symmetric hormonal alopecia in a male collie showing signs of feminization secondary to a functional Sertoli cell tumor. *(Photograph courtesy Dr. Karen Campbell.)*

often are older, and complete clinical staging before surgical intervention is recommended. A thorough physical examination with rectal palpation should be performed to detect regional lymph node enlargement; the prostate gland also should be palpated. Preoperative clinical staging at least should include a complete blood count to screen for hematologic abnormalities associated with hyperestrogenism, such as leukopenia, thrombocytopenia, and anemia.

An abdominal ultrasound scan often is useful for identifying undescended, nonpalpable testicles in the abdominal cavity or inguinal canal and to assess the regional lymph nodes and evaluate for distant metastasis and prostatic changes secondary to hormonal imbalances. Regional and distant metastatic disease can be confirmed with fine-needle aspiration cytology or histopathologic evaluation. Testicular ultrasonography also can be useful for differentiating neoplastic conditions from nonneoplastic disorders (e.g., orchitis, epididymitis, and testicular torsion).[37] In dogs with signs of feminization, measurement of plasma estradiol-17β levels may support the diagnosis of hyperestrogenism secondary to a functional primary testicular tumor. However, not all dogs with signs of feminization have an absolute increase in the estradiol-17β level; rather, clinical signs may be attributed to a relative imbalance (decrease) in the androgen/estrogen ratio.[26]

Therapy and Prognosis

As mentioned previously, most primary testicular tumors are locally confined, with fewer than 15% having a metastatic phenotype. In dogs with localized
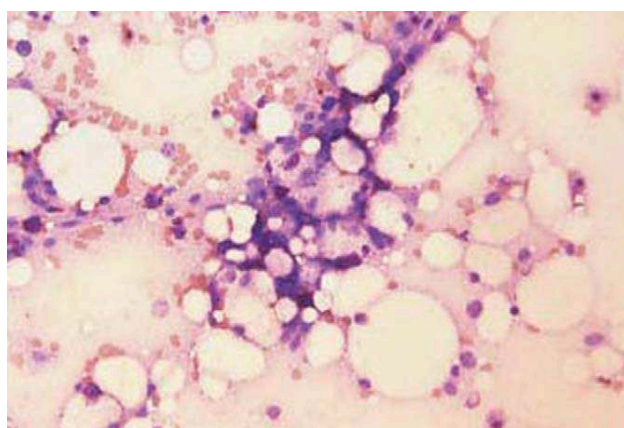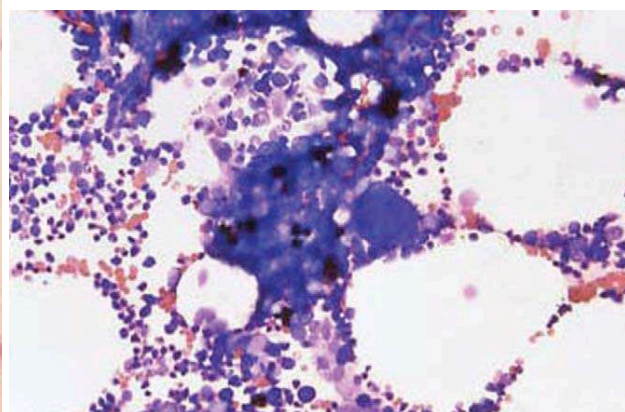
A   B

Figure 27-4

Hypoplastic bone marrow with minimal precursor cells **(A)** and normal canine bone marrow **(B).** The severe bone marrow hypoplasia occurred secondary to hyperestrogenism associated with a functional canine Sertoli cell tumor. (Wright's stain, 400× magnification.) *(Photograph courtesy Dr. Anne Barger.)*

disease, orchiectomy with scrotal ablation remains the treatment of choice and often is curative. Unilateral orchiectomy can be considered in a valuable breeding animal. Dogs with bone marrow hypoplasia secondary to hyperestrogenism have a guarded prognosis because of the high morbidity and mortality associated with either secondary infection or bleeding diathesis. In such critically ill patients, intensive supportive care with blood products and antibiotics may be required for several weeks before any signs of bone marrow recovery appear.[30]

Primary testicular tumors occasionally metastasize to regional lymph nodes and distant sites, including the skin, lungs, liver, and spleen. Information about appropriate and effective management of metastatic disease is limited, although the use of radiation therapy and systemic chemotherapy has been reported. In one report, four dogs with metastatic seminoma confined to the regional lymph nodes were treated with [137]cesium teleradiotherapy.[38] The total dose of radiation varied among patients, ranging from 17 to 40 Gy. All four dogs showed tumor regression, and three of the dogs survived longer than 37 months. The results of this small case series suggest that regionally confined metastatic seminomas may be managed effectively with external beam radiation therapy. Systemic chemotherapy for the treatment of metastatic Sertoli cell tumors and seminomas also has been reported. However, given the small number of dogs treated, the efficacy of chemotherapeutic agents, including cisplatin and bleomycin, for the management of metastatic testicular tumors cannot be firmly stated.[20,21] Overall the prognosis for dogs with distant metastatic disease is variable, with the survival time of dogs treated with systemic chemotherapy ranging from 5 months to longer than 31 months.

Comparative Aspects

In men, the incidence of germ cell tumors in cryptorchid testes is several times higher than in normally descended testes.[39] However, additional factors that may increase the risk of testicular tumor development in men include maternal exposure to diethylstilbestrol and Klinefelter's syndrome. Unlike their canine counterpart, most human testicular tumors arise in young men 20 to 35 years of age. In men, half of all germ cell tumors arising from the testes are seminomas and can be histologically categorized, based on their mitotic index, as either classical or anaplastic. Spermatocytic seminoma is a rare histologic variant in men that has a low metastatic potential similar to that of canine testicular tumors. Although testicular tumors in men generally are metastatic, the prognosis is favorable; approximately 70% to 80% of patients are cured with cisplatin-based chemotherapy. However, systemic chemotherapy is not always indicated; orchiectomy in conjunction with either radiation therapy

to regional lymph nodes or observation is considered an effective treatment option for men diagnosed with stage I seminomas.[40,41]

CANINE PROSTATIC NEOPLASMS

Incidence

Prostatic cancer is an uncommon canine malignancy, having a prevalence of 0.2% to 0.6% based on necropsy studies.[42,43] Despite its low and sporadic incidence, the dog is one of the few large mammalian species in which spontaneous prostatic cancer occurs. Although the etiology of canine prostatic cancer is unknown, prostatic intraepithelial neoplasia (PIN), a precursor lesion of prostatic carcinoma, has been described in both normal and cancerous canine prostate glands.[44-46] The identification of PIN in dogs may parallel the multistep carcinogenic process observed in men diagnosed with prostatic carcinoma. In dogs, most prostatic tumors are epithelial in origin, and adenocarcinoma is the most common type. Additional histologic subtypes include squamous cell carcinoma, transitional cell carcinoma, and undifferentiated carcinomas.[43,47] Tumors of mesenchymal origin (e.g., hemangiosarcoma, leiomyosarcoma) also can affect the canine prostate gland, although these have a much lower incidence.[48,49] Prostatic carcinoma occurs most frequently in elderly dogs (median age at diagnosis, 10 years).[43,50]

Risk Factors

Both sexually intact and castrated dogs can develop prostatic carcinoma, and the influence of androgens in the development and biologic behavior of neoplastic prostatic diseases remains controversial. One report suggested that castration at any time before the formation of prostatic carcinoma did not provide any protective effect in dogs.[42] However, the findings of another study suggest that castration before sexual maturity (at about 6 months of age) reduces the risk of prostatic carcinoma.[47] In direct contrast, further investigations suggest that castration may increase the incidence and biologic aggressiveness of canine prostatic carcinoma. In one study, castrated dogs diagnosed with prostatic carcinoma were more likely to develop pulmonary metastasis than were intact dogs with the same disease.[43] Furthermore, castrated dogs in two recent studies were at an increased risk for prostatic adenocarcinoma, with odds ratios ranging from 3.9 to 4.34.[51,52]

Canine prostatic carcinomas arise from ductal epithelium, which is predominantly androgen receptor negative. The absence of androgen receptors on neoplastic ductal epithelial cells suggests that androgens may

not be required for initiation or progression of canine prostatic adenocarcinoma.[52,53] Whether androgens exert a protective or permissive effect for the formation of canine prostatic carcinoma remains to be definitively determined. Prospective studies comparing the castration status and duration of androgen exposure of dogs diagnosed with prostatic carcinoma and age-matched controls would be useful for determining whether canine prostatic carcinoma behaves dependently or independently of androgen stimulation.

Pathology and Natural Behavior

Most canine prostatic carcinomas are locally invasive and metastatic. In one study, 80% of dogs had evidence of gross metastatic disease at necropsy, and regional lymph nodes and lungs were the most common sites of metastasis.[47] Canine prostatic carcinoma also has a predilection for skeletal metastasis; 22% to 42% of patients showed neoplastic involvement of the axial skeleton.[47,54] Recent investigations have tried to identify mechanisms that may contribute to the aggressive clinical phenotype associated with canine prostatic carcinoma.

In humans, epidemiologic and experimental evidence supports the use of nonsteroidal antiinflammatory drugs (NSAIDs) for the prevention of cancer development. Mechanistically, this apparent chemopreventive effect may be partly mediated through the inhibition of cyclooxygenase-2 (COX-2) activity causing the blockade of endogenous prostaglandin E_2 production. Although prostaglandin E_2 is involved in normal physiologic functions, it also exerts pro-oncogenic effects, including cell proliferation, migration, evasion of apoptosis, decreased immune surveillance, and increased angiogenesis. In one study, expression of the COX-1 and COX-2 proteins was evaluated in normal canine prostates and prostatic carcinomas.[55] Normal prostatic tissue failed to express the COX-2 protein, but it was detected in 18 of 24 (75%) prostatic carcinomas. In contrast, COX-1 was expressed in all normal and neoplastic samples, localized in stromal fibroblasts and vascular endothelium. The identification of the COX-2 protein in most prostatic carcinomas, in conjunction with its absence in normal prostate gland, suggests that COX-2 expression may be involved in the development of canine prostatic carcinoma.[55] In support of the pro-oncogenic effects of COX-2 expression in canine prostatic carcinoma, a recent clinical study demonstrated COX-2 protein expression in 88.2% of prostatic carcinomas and also identified a survival advantage in dogs treated with COX-2 inhibitors.[56]

As mentioned previously, prostatic carcinomas have a predilection for skeletal metastasis. Several *in vitro* studies have investigated possible mechanisms to account for this unique metastatic pattern. The successful establishment and growth of cancer cells in the bone microenvironment involves tumor-derived factors such as transforming growth factor beta (TGF-β) and parathyroid hormone– related peptide (PTHrP). The active role of PTHrP in mediating pathologic bone resorption has been demonstrated in several tumor models, and cytokines that upregulate PTHrP gene transcription would promote the development of skeletal metastases.[57] TGF-β is liberated directly from bone during resorptive processes and also can be liberated directly by certain cancer cells. *In vitro* studies using canine prostatic carcinoma cells have demonstrated that the addition of exogenous TGF-β increases gene transcription for PTHrP in carcinoma cells.[58] These laboratory findings suggest that canine prostatic carcinoma cells have the ability to induce the bone resorption necessary for the establishment of skeletal metastases through a regulatory pathway potentially involving TGF-β and PTHrP gene transcription.

Although PTHrP may be involved in pathologic bone resorption, prostatic carcinoma skeletal metastases are most commonly osteoproductive in nature. The mechanism involved in the formation of osteoblastic metastases recently has been investigated *in vitro*. Osteoblast activation, as assessed by increases in the activity of bone alkaline phosphatase (ALP), was increased in a rat calvaria bone model after incubation with normal canine prostate protein homogenates.[59] An endothelin-dependent mechanism was the identified pathway involved in osteoblast activation subsequent to canine protein homogenate incubation. Whether endothelin-dependent mechanisms are operative and contribute to the formation of osteoblastic skeletal metastases in naturally occurring canine prostatic carcinoma remains to be defined.

History and Clinical Signs

Clinical signs of prostatic cancer in dogs are variable and can be attributed to local or distant disease progression. Upon rectal palpation, the prostatic gland may be enlarged, nodular, asymmetric, firm, nonmovable, and/or painful. Dysuria is common, and macroscopic hematuria may be evident. With prostatic enlargement, compression or invasion of the urethra by neoplastic cells may prevent complete voiding of urine, predisposing the dog to the development of pyuria, bacteriuria, stranguria, and dysuria. Complete obstruction of urinary outflow may result in hydroureter, hydronephrosis, and subsequent renal failure. In addition to urinary signs, some dogs may experience tenesmus. Flattened or ribbonlike stools can result from concurrent dorsal and ventral compression of the rectum caused by enlargement of the sublumbar lymph nodes or prostate gland, respectively. In addition to physical obstruction, local invasion of prostatic carcinoma into the lumbar vertebrae or nerve roots may result in constipation or gait abnormalities secondary to osteolytic or neuropathic pain or neurologic compromise.

Given the high metastatic potential of canine prostatic carcinoma, dogs with advanced disease may have nonspecific signs of illness, including lethargy, anorexia, weight loss, and malaise. Dogs with diffuse pulmonary metastasis may be exercise intolerant and, with advanced disease, tachypneic or dyspneic. Canine prostatic carcinoma commonly metastasizes to bone, and dogs may exhibit signs of acute and excruciating osteolytic pain. Bony metastases usually involve the axial skeleton (e.g., lumbar vertebrae and pelvis), although in some cases the proximal portions of the femur or tibia are affected.[43,54,60] Skeletal metastases often are osteoblastic, a radiographic pattern most consistently associated with prostatic carcinoma in both humans and dogs.

Diagnostic Techniques and Workup

Dogs suspected of having prostatic neoplasia should be completely staged to determine the extent of disease. Complete blood counts and serum chemistry panels may demonstrate evidence of anemia, leukocytosis, hypercalcemia (secondary to extensive bony metastasis), and elevations in bone ALP activity. Thoracic radiographs are useful for detecting pulmonary metastases and sternal lymphadenopathy (Figure 27-5). When sonographic equipment is not available, survey abdominal radiographs may allow identification of prostatomegaly, sublumbar lymphadenopathy, and bone metastasis. Mineralization of the prostatic parenchyma, depicted radiographically, is suggestive of prostatic carcinoma. When clinically indicated, survey radiographs or bone scintigraphy may allow localization of suspect bony metastases (Figure 27-6). Abdominal ultrasound can provide information about the size of the prostate and any involvement of regional lymph nodes and visceral organs. Definitive diagnosis of prostatic neoplasia requires either cytologic or histologic confirmation. Samples for examination can be obtained through closed (percutaneous, transrectal, and perineal) or open (laparotomy) surgical approaches. Cytologic evaluation of samples collected either with traumatic catheterization or a prostatic wash may yield a diagnosis of carcinoma (Figure 27-7).

Therapy and Prognosis

Given the high metastatic rate and insidious nature of canine prostatic carcinoma, most dogs are diagnosed at an advanced stage of disease. The prognosis and long-term
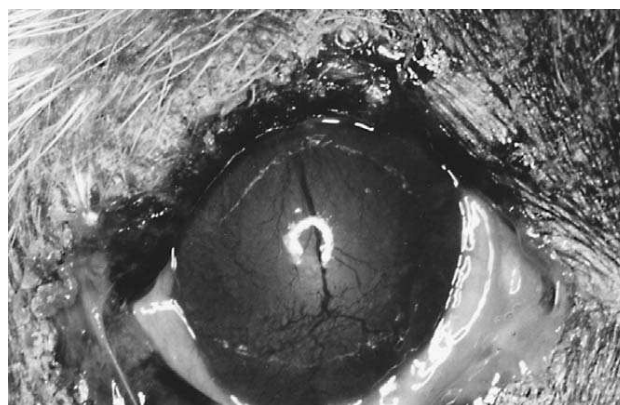
Figure 27-6

Radiograph from a dog that had lumbar pain and hind limb weakness. The patient was diagnosed with prostatic carcinoma and a cytologically confirmed osteoblastic metastatic lesion involving lumbar vertebra 3.

Figure 27-5

Thoracic radiographs of a dog with prostatic carcinoma and distant metastases to the pulmonary parenchyma, which were diagnosed upon clinical staging. This dog also had cytologically confirmed regional lymph node metastasis.
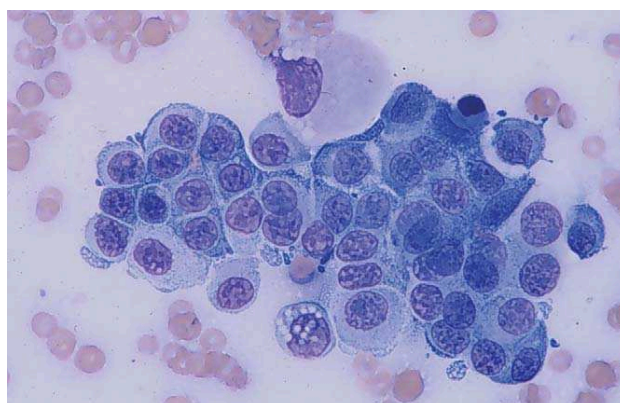
Figure 27-7

Cytologic preparation from a dog with a prostatic carcinoma. Malignant epithelial cells demonstrate moderate anisocytosis, anisokaryosis, and prominent multiple nucleoli. (Wright's stain, 400× magnification.)

survival are guarded for most patients. Treatment of dogs with prostatic carcinoma should address both local and distant disease. Therapeutic options for managing local disease include surgical prostatectomy, radiation therapy, photodynamic therapy (PDT), and electrosurgical transurethral resection (TUR) in conjunction with intraoperative radiation therapy (IORT).

Surgical prostatectomy is associated with a high rate of complications, including urinary incontinence; however, it may be appropriate in dogs with early stage disease still confined within the prostatic capsule.[61,62]

Palliative radiation is a reasonably effective treatment option for dogs with significant localized tumor burden. In one study, 10 dogs with prostatic carcinoma were treated with palliative intraoperative orthovoltage radiation therapy. Nine of the 10 dogs received a total dose of 20 to 30 Gy, and the median survival time for these dogs was 114 days.[63] The tenth dog received an intraoperative fraction of 15 Gy, and then a boost of 40 Gy of megavoltage radiation using cobalt-60.

PDT has been used to treat minimally invasive canine prostatic carcinoma. In one descriptive case report, PDT resulted in resolution of macroscopic hematuria and provided stable disease for at least 24 weeks in a dog with prostatic carcinoma.[64]

Minimally invasive surgical techniques for palliative treatment of canine prostatic carcinoma recently have been described. In a case series report, electrosurgical TUR with or without IORT was used in three dogs with either prostatic transitional cell carcinoma or undifferentiated carcinoma.[65] Clinical signs associated with urethral obstruction resolved rapidly in all three dogs after TUR. However, severe complications, including urethral perforation, were observed in two of the three dogs. In addition, the sole palliative effect of TUR with IORT could not be evaluated in all patients because of concurrent placement of cystostomy tubes or adjuvant use of either piroxicam or mitoxantrone.[65] Two recent studies evaluated novel palliative surgical techniques for canine prostatic carcinoma. One study described a partial prostatectomy technique with Neodymium: Yttrium Aluminum Garnet (Nd:YAG) laser in eight dogs.[1] The overall median survival time in all eight dogs was 103 days, although three dogs died from complications within 16 days post-operatively. The second study evaluated the palliative stenting of the urethra in 12 dogs, including 8 dogs with prostatic tumors (7 carcinomas, and 1 osteosarcoma).[2] Stenting resulted in a good to excellent outcome in six of the eight dogs with prostatic tumors, and complications encountered in 2 of them were mild. In addition, 6 of the 8 dogs were either fully urinary continent, or had mild incontinence following the stenting procedure, and no male dog died or was euthanized because of urinary tract obstruction.[2]

Treatment of metastatic prostatic carcinoma with systemic chemotherapy or supportive therapies remains poorly defined in veterinary oncology. For dogs with

Figure 27-8

Low-profile cystostomy tube placed in a beagle with urethral obstruction caused by a prostatic carcinoma.

urethral obstruction, placement of a cystostomy tube may enhance the quality of life and extend the overall survival time (Figure 27-8). Although some benefit may be derived from the use of antineoplastic agents to manage pulmonary or visceral metastases, prospective studies evaluating the effectiveness of chemotherapeutic protocols have not been published. Similarly, the management of painful skeletal metastases with palliative radiation therapy, Samarium-153 ethylene diamine tetramethylene phosphonate (^{153}Sm-EDTMP), or intravenous aminobisphosphonates appears reasonable, but standardized and effective palliative protocols have not been documented.

Although information is limited, a recent study evaluated the usefulness of NSAIDs in the management of canine prostatic carcinoma.[56] In 35 dogs diagnosed with prostatic carcinoma, 16 dogs were treated with piroxicam (n = 14) or carprofen (n = 2), 15 dogs did not receive any specific therapy for their cancer and four dogs were excluded from survival analysis because of confounding factors. The dogs that received an NSAID had a median survival time of 6.9 months, whereas those that did not receive any specific cancer therapy had a median survival time of only 0.7 months.[56] Although these results are promising, additional prospective clinical trials are necessary to better define the true efficacy of NSAIDs for the management of prostatic epithelial malignancies when used alone or in combination with standard cytotoxic chemotherapy.

Comparative Aspects

Given its high incidence and mortality, prostate cancer is the leading cause of cancer-related death in American men. Most prostatic tumors are adenocarcinomas.

Similar to the case in dogs, prostatic carcinoma in men is biologically aggressive and shows early lymphatic and vascular invasion. In men, malignant transformation of prostatic acinar cells is believed to be a multistep process, and androgen stimulation appears to play a significant role in the pathogenesis of tumor development. Men diagnosed with prostatic carcinoma commonly have additional, premalignant lesions called *prostatic intraepithelial neoplasia (PIN)*. Similar PIN-like lesions have been identified in normal and malignant canine prostates, which suggests that similar carcinogenic pathways may lead to the development of advanced-stage prostatic carcinoma in humans and canines. Prostatic carcinoma predominantly affects elderly men (African-American men have an increased risk), and the overall incidence of prostatic carcinoma is far higher in humans than in canines. Based on necropsy studies, the incidence of prostatic carcinoma in dogs is estimated to be 0.2% to 0.6%.[42,43] In humans, it is estimated that one in seven men between the ages of 60 and 79 years will develop clinically apparent disease.

Another difference between man and dog is the role of androgens in the pathogenesis of prostatic carcinoma. In men, the prostatic acinar cell is androgen responsive and undergoes malignant transformation. In the dog, prostatic carcinoma appears to arise from the ductal or urothelial epithelium, which is not driven to proliferate subsequent to androgen stimulation. Given the androgen dependence of prostatic carcinoma in men, androgen deprivation remains the cornerstone of treatment for metastatic disease. The availability and routine measurement of an accurate surrogate marker of prostatic disease, serum prostate-specific antigen (PSA), a serine protease produced by benign and malignant prostate tissues, has allowed early detection of locally confined prostatic carcinoma in men. With early detection, men diagnosed with prostatic carcinoma have a 5-year survival rate of 90%. Unfortunately, serum PSA in dogs is not detectable with commercially available human antibodies, which limits early disease detection in susceptible male dogs.[66] Given the shared predilection for osteoblastic skeletal metastases in men and dogs, therapies that are effective for managing pathologic bone events in men with prostatic carcinoma (e.g., intravenous aminobisphosphonates) may also have therapeutic efficacy in the treatment of canine skeletal metastases.

TUMORS OF THE CANINE PENIS, OS PENIS, AND PREPUCE

Many tumors can affect the epithelial surface of the canine penis and haired prepuce, including squamous cell carcinomas, transmissible venereal tumors (TVTs), mast cell tumors, lymphomas, and extramedullary plasmacytomas.[67-70] Ossifying fibroma and osteosarcoma

Figure 27-9

Grade III preputial cutaneous mast cell tumor in a Boston terrier, characterized by aggressive primary disease and regional (inguinal) lymph node involvement.

are two tumor types reported to affect the canine os penis.[71,72] The most common tumor affecting the canine penis is TVT, which is discussed elsewhere in this text. Tumors of the canine penis, os penis, and prepuce typically cause urinary signs, such as stranguria, dysuria, and hematuria. Although clinical manifestations often are associated with progression of local disease, some tumors may have aggressive characteristics, allowing neoplastic cells to establish in regional lymph nodes and distant sites (Figure 27-9). Except for TVTs, the biologic behavior of most penile tumors is poorly described. For this reason, dogs with penile tumors should be thoroughly staged to assess the extent of local and distant disease. For localized tumors, treatment involves partial or complete penile amputation and perineal urethrostomy. Adjuvant therapies for regional or distant metastatic disease may be beneficial with certain tumors.

Comparative Aspects

Carcinoma of the human penis is rare, although circumcision appears to be protective.[73] In circumcised men the incidence of penile carcinoma is less than 2%, compared to 10% to 12% in uncircumcised men. The increased incidence of penile carcinoma in uncircumcised men is believed to result from the combined effects of poor hygiene and the irritant properties of smegma, the product of bacterial action on desquamated epithelium. Most penile carcinomas are of squamous cell origin; other histologic subtypes (e.g., melanoma and basal cell carcinoma) are infrequently diagnosed. The most common manifestation of penile cancer is a mass

or persistent sore involving the glans, foreskin, or shaft. Most localized penile tumors can be managed successfully with surgical resection and excision of the regional lymph nodes. Radiation therapy and systemic chemotherapy may be used when surgery alone is not curative.

TUMORS OF THE FELINE MALE REPRODUCTIVE SYSTEM

Feline Testicular Tumors

Feline testicular tumors are rare. Reported tumor types include Sertoli cell tumor, interstitial cell tumor, teratoma, malignant seminoma, and a possible rete testis tumor.[74-78] In the few reported cases in cats, at least three patients were 2 years old or younger at diagnosis.[74,76,78] The true biologic behavior of feline testicular tumors is unknown, although metastatic disease has been described.

Feline Prostatic Tumors

Feline prostatic tumors also are rare. Eight cases can be found in the veterinary literature in English, and most of these were adenocarcinomas.[74,79-82] Cats with prostatic carcinomas tend to be older (median age at presentation, 10.5 years; range, 6 to 22 years), and in the studies mentioned, all but one had been castrated. However, the rarity of the condition precludes conclusions about an increased risk associated with castration.

In the reported cases, the clinical signs and history included lower urinary tract signs occasionally accompanied by dyschezia or obstipation. Rectal palpation revealed a mass in most cases. The diagnosis is obtained in a manner similar to that described for canine prostatic tumors, with a combination of imaging and histopathologic evaluation. As with feline testicular tumors, definitive information on prognostic factors and the tumor's biologic behavior can hardly be extrapolated from the few reported cases. Metastases were reported in three of the eight cats described, and most of the cats died within 3 months of diagnosis.[79-82] Surgical resection and chemotherapy have been attempted, but the true benefit in survival time or quality of life remains unknown.

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CHAPTER 28

Tumors of the Urinary System

Deborah W. Knapp

CANINE URINARY BLADDER TUMORS

Urinary bladder cancer accounts for approximately 2% of all reported malignancies in the dog.[1,2] Invasive transitional cell carcinoma (TCC) is the most common form of canine urinary bladder cancer.[1-3] With more than 60 million dogs in the United States, even uncommon forms of cancer strike thousands of dogs each year.[2] The hospital prevalence or proportionate morbidity of bladder cancer at university-based veterinary hospitals is increasing.[2] Most TCCs are intermediate- to high-grade papillary infiltrative tumors.[2,3] Other types of bladder tumors reported less frequently include squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, rhabdomyosarcoma, fibroma, and other mesenchymal tumors.[3,4]

The etiology of canine bladder cancer most likely is multifactorial. Identified risk factors include exposure to topical insecticides and herbicides, obesity, possibly cyclophosphamide administration, female gender, and breed (Table 28-1).[1,2,5-7] Multiple studies have confirmed the increased risk of bladder cancer in female dogs.[1,2,4] The female to male ratio in a series of 102 dogs with TCC treated at the Purdue University Veterinary Teaching Hospital (PUVTH) was 1.7:1.[1,2] Similarly, in a report of 115 dogs with bladder or urethral tumors, the female to male ratio was 1.95:1.[4]

An association between herbicide exposure and TCC in dogs was identified in a case control study of 166 Scottish terriers.[6] Exposure to herbicides was compared between TCC patients (n = 83) and a group of Scottish terrier control dogs (n = 83) at least 6 years old with no evidence of TCC and no history of urinary tract disease in the 2 years before entry into the study. The owners of the case and control dogs completed a written questionnaire on the dog's medical history and exposure to household lawn or garden chemicals for 1 year before diagnosis for the cases and for a comparable period for the control dogs. The risk of TCC was significantly increased in dogs that had been exposed to lawns or gardens treated with herbicides alone (Odds ratio [OR], 3.62; 95% confidence interval [CI] 1.17-11.19)

or gardens treated with herbicides and insecticides (OR, 7.19; 95% CI 2.15-24.07), but it was not significantly increased for dogs exposed to lawns or gardens treated with insecticides alone (OR, 1.62; 95% CI 0.56-4.74).[6] Important gene-environment interactions likely were involved in the development of TCC, and limiting dogs' exposure to herbicides, especially breeds at increased risk for these tumors, is an appropriate measure.

An earlier case control study revealed an association between TCC and exposure to topical application of flea and tick dips.[5] In the highest risk group (overweight female dogs), the risk of TCC was 28 times that of normal-weight male dogs not exposed to the insecticides.[5] The authors speculated that the "inert" ingredients (solvents and petroleum distillates), which accounted for more than 95% of the product, were the probable carcinogens. Newer, spot-on types of flea control products appear safer. In a recent case control study in Scottish terriers, spot-on products containing fipronil were not associated with an increased risk of TCC.[7]

Ingestion of vegetables recently has been associated with a lower risk of TCC.[8] In a case control study, Scottish terriers that ate vegetables at least three time a week, along with their normal diet, had a reduced risk of TCC (OR, 0.30; 95% CI 0.01-0.97).[8]

TCC most often is found in the trigone region of the bladder. Papillary lesions and a thickened bladder wall (Figure 28-1) are common features and can lead to partial or complete urinary tract obstruction. In a series of dogs with TCC examined at the PUVTH, the tumor involved the urethra as well as the bladder in 57 of 102 dogs (56%), and it involved the prostate in 11 of 38 (29%) male dogs.[2] Lymph node metastasis and distant metastasis were present in 16% and 14% of 102 dogs, respectively, at the time of diagnosis of TCC. Distant metastasis was detected in 50% of the dogs at the time of death. Using the clinical staging system for canine bladder tumors established by the World Health Organization (WHO) (Box 28-1), 78% of the 102 dogs had T_2 tumors, and 20% had T_3 tumors, as staged by ultrasonography and cystography and/or biopsy.[2,9]

TABLE 28–1	Breed and Risk of Bladder Cancer in Pet Dogs*	
Breed	**Odds Ratio**	**95% Confidence Interval**
Mixed breed	1.0[†]	—
All purebreds	0.74	0.62-0.88
Scottish terrier	18.09	7.30-44.86
Shetland sheepdog	4.46	2.48-8.03
Beagle	4.15	2.14-8.05
Wirehaired fox terrier	3.20	1.19-8.63
West Highland white terrier	3.02	1.43-6.40
Miniature schnauzer	0.92	0.54-1.57
Miniature poodle	0.86	0.55-1.35
Doberman pinscher	0.51	0.30-0.87
Labrador retriever	0.46	0.30-0.69
Golden retriever	0.46	0.30-0.69
German shepherd	0.40	0.26-0.63

Modified from Knapp DW, Glickman NW, DeNicola DB et al: Naturally-occurring canine transitional cell carcinoma of the urinary bladder: a relevant model of human invasive bladder cancer, Urol Oncol 5:47-59, 2000.

*This table represents a summary of data from 1290 dogs with transitional cell carcinoma (TCC) and 1290 institution and age-matched control dogs without TCC in the Veterinary Medical Data Base.

[†]Reference category.

Figure 28-1

Papillary invasive transitional cell carcinoma observed in the urinary bladder of a dog during a postmortem examination. *(Slide courtesy T. Lin.)*

Box 28-1	TNM Clinical Staging System for Canine Bladder Cancer

T—Primary tumor

T_{is} Carcinoma *in situ*
T_0 No evidence of a primary tumor
T_1 Superficial papillary tumor
T_2 Tumor invading the bladder wall, with induration
T_3 Tumor invading neighboring organs (prostate, uterus, vagina, and pelvic canal)

N—Regional lymph node (internal and external iliac lymph node)

N_0 No regional lymph node involvement
N_1 Regional lymph node involved
N_2 Regional lymph node and juxtaregional lymph node involved

M—Distant metastases

M_0 No evidence of metastasis
M_1 Distant metastasis present

Modified from Owen LN: TNM classification of tumours in domestic animals, Geneva, 1980, World Health Organization.

Diagnosis and Clinical Staging

Common clinical signs in dogs with TCC include hematuria, dysuria, pollakiuria and, less commonly, lameness caused by bone metastasis or hypertrophic osteopathy.[1] Signs may be present for weeks to months and may resolve temporarily with antibiotic therapy. A physical examination, which includes a rectal exam, may reveal thickening of the urethra and trigone region of the bladder, enlargement of iliac lymph nodes, and sometimes a mass in the bladder or a distended bladder. However, normal findings on a physical examination do not rule out TCC.

When TCC is suspected, the evaluation should include a complete blood count (CBC), serum biochemistry profile, urinalysis, urine culture, radiography of the thorax and abdomen, and bladder imaging (contrast cystography or cystosonography) (Figures 28-2 and 28-3).[10] To avoid the risk of seeding TCC through cystocentesis, urine may be collected by free catch or catheterization.[11] Care must be taken when a urinary catheter is passed during diagnostic procedures to avoid penetrating the diseased bladder or urethral wall. With cystosonography, it is important that the bladder be distended; this is best accomplished by instilling 4 to 8 ml/kg of sterile saline into the bladder through a urinary catheter. For subsequent examinations on the patient, the same degree of distention should be used to ensure accurate comparison of tumor sizes between studies; this is achieved by instilling the known amount of saline into the bladder via a catheter.
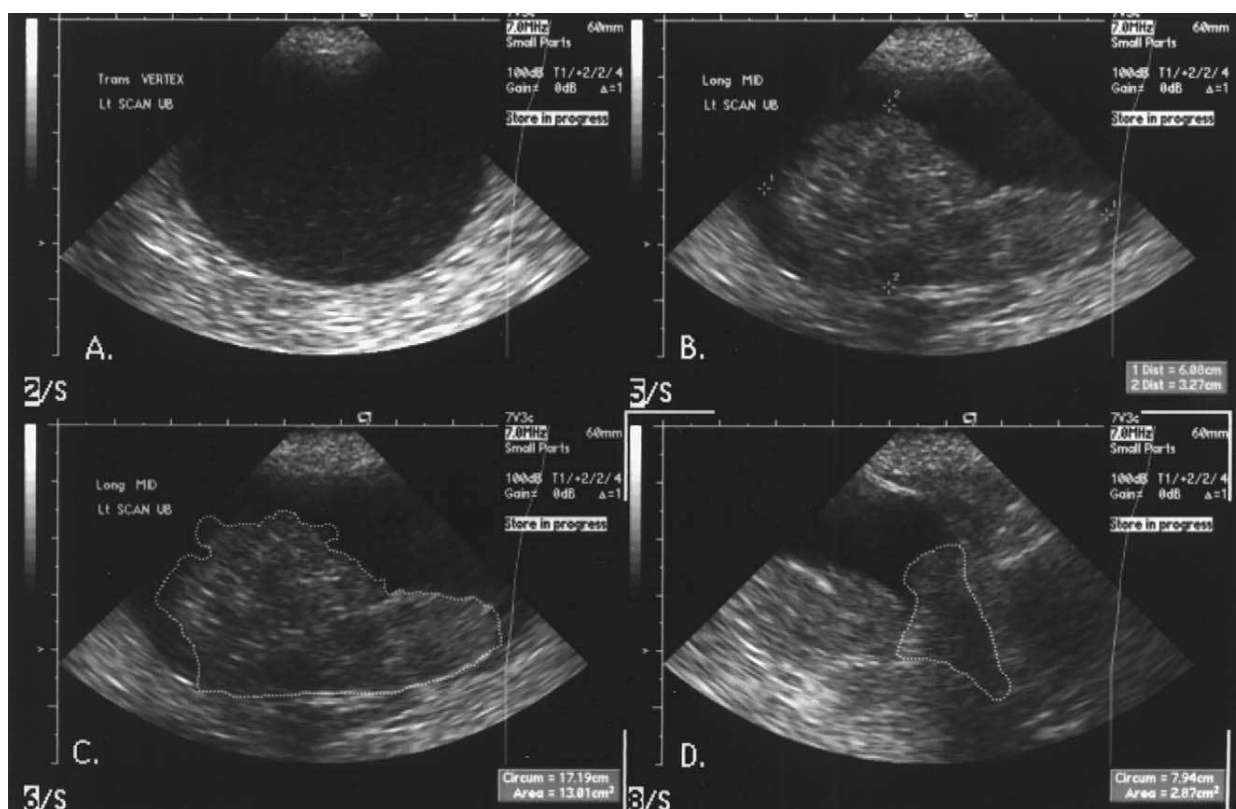
Figure 28-2

Cystosonography of a dog with transitional cell carcinoma. No masses are present in the vertex of the bladder **(A)**. Masses are observed in the midbody **(B** and **C)** and in the trigone region, extending into surrounding tissues **(D)**. For the most accurate localization and measurement of bladder masses, cystosonography should be performed with the bladder distended with urine or infused saline and distended with a similar volume for each subsequent examination. *(Ultrasound images courtesy W. Widmer.)*

Ultrasonography also is useful for viewing the regional lymph nodes and metastases in other abdominal organs.

A diagnosis of TCC requires histopathologic confirmation. Although neoplastic cells may be present in the urine of 30% of dogs with TCC,[4] neoplastic cells often are indistinguishable from reactive epithelial cells associated with inflammation. Urine antigen tests for TCC have been found to be sensitive,[12] but a high number of false positive results limits the value of these tests. A bladder or urethral mass may raise suspicion of TCC, but other conditions also can cause bladder and urethral masses (Figure 28-4), such as granulomatous cystitis/urethritis, polypoid cystitis, benign tumors, and other types of malignant tumors. Therefore histopathologic examination of the abnormal tissues is essential to determine whether TCC is present. Methods of obtaining tissue for histopathologic diagnosis include cystotomy, cystoscopy, and traumatic catheterization.[2] Percutaneous biopsy methods can lead to tumor seeding and are generally avoided.[11]

TCC infrequently can metastasize to bone. A bone scan may be considered if lameness is present that is not easily explained by common orthopedic or neurologic disease. Often a side-by-side comparison of radiographs and a nuclear bone scan are necessary to rule out (or in) a bone metastasis.

Treatment

Surgery

Complete surgical excision of TCC usually is not possible because of the typical trigonal location and the urethral involvement of the tumor. In addition, many dogs appear to develop multifocal TCC in the bladder. This is consistent with the "field effect" proposed in human patients with bladder cancer in which the entire bladder lining is thought to undergo malignant change in response to exposure to carcinogens in the urine.[13] In a series of 67 dogs with TCC that underwent surgery for biopsy or for therapeutic intent, complete surgical excision of the tumor with tumor-free margins was possible in only two dogs.[2] One of the two dogs had a relapse in the bladder 8 months later, and the second dog developed metastatic disease.

Patients with tumors "apparently" located away from the trigone and urethra (i.e., bladder apex) may be the best candidates for partial cystectomy. Ureteral divergence surgery (i.e., ureterocolonic anastomosis) was reported in 10 dogs with obstructive TCC, but the complications rate was high, and none of the dogs survived longer than 5 months after surgery.[14] Transurethral resection of urethral tumors has been attempted but has not been successful because of advanced local disease, complications of the procedure, and local disease recurrence.[15]

Surgery has been used as an emergency, palliative procedure to debulk nonresectable tumors in dogs with distal urinary tract obstruction. Prepubic cystostomy catheters that bypass a urethral obstruction also have been used in a small number of dogs.[16] Cystotomies do not benefit patients with bilateral ureteral obstruction.

Radiation therapy

Whole bladder intraoperative radiation therapy has been evaluated in dogs with TCC and other bladder tumors.[17,18] In one report, only one of seven dogs treated with radiation was alive 1 year after treatment.[18] In another report, which involved 13 dogs, the 1- and 2-year survival rates were 69% and 23%, respectively, but complications of therapy (i.e., urinary incontinence and cystitis with accompanying pollakiuria and stranguria) detracted from the patients' quality of life.[17] Other studies have confirmed the complications associated with pelvic irradiation.[19] A report of 10 dogs that received weekly coarse fraction external beam radiation therapy, along with mitoxantrone and piroxicam chemotherapy, indicated that this regimen was well tolerated; however, it showed no improvement on results obtained with mitoxantrone-piroxicam therapy without radiotherapy.[20]

Medical management of TCC is indicated in dogs with nonresectable or metastatic tumors and as a postoperative adjuvant in the rare patient that undergoes resection.

Medical therapy

Medical therapy of TCC has consisted of chemotherapy, cyclooxygenase (COX) inhibitors (nonselective cox

A

B
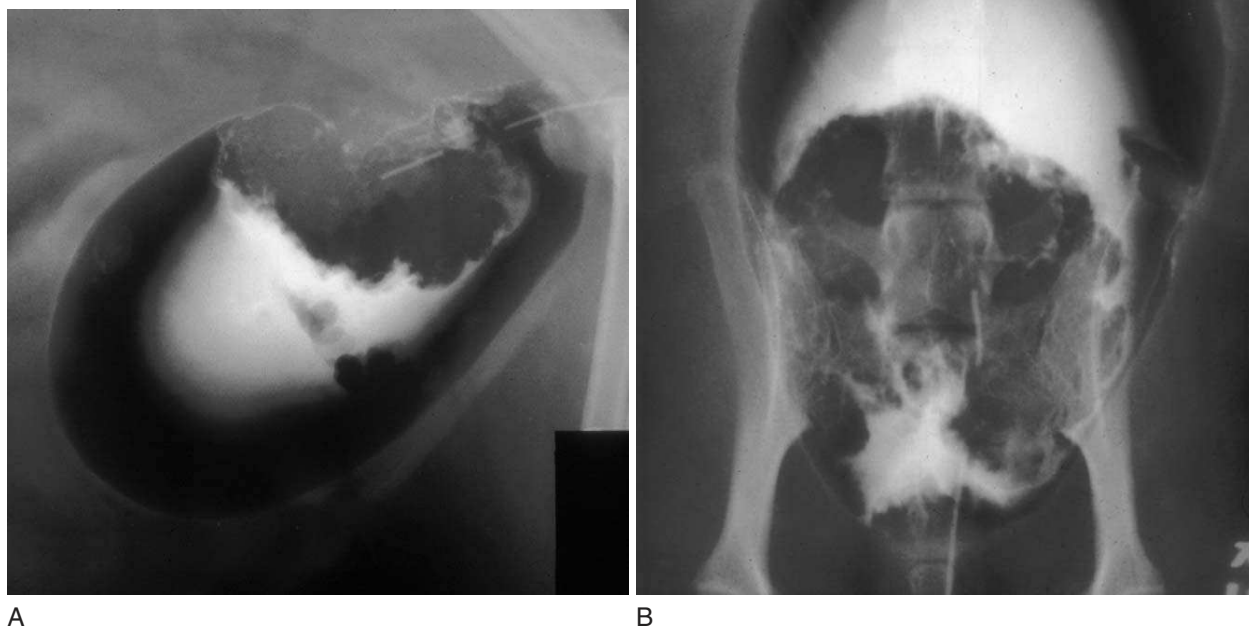
Figure 28-3

Double-contrast cystogram of a dog with transitional cell carcinoma of the urinary bladder. Note the large, irregular filling defect in the trigone region of the bladder on lateral **(A)** and ventrodorsal **(B)** views. *(Radiographic images courtesy W. Widmer.)*

inhibitors and COX-2 inhibitors[1,2,10,21-27]), and combinations of these two types of drugs (Table 28-2).[1,2,10,18-24]

The treatment that has resulted in the highest percentage of dogs in remission is cisplatin combined with piroxicam; however, this particular combination is not
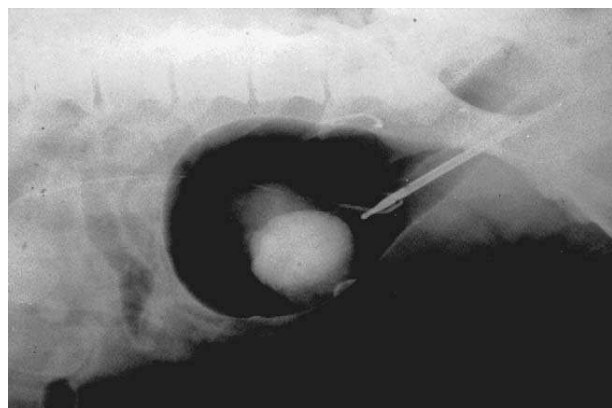
Figure 28-4

Double-contrast cystogram of an 11-year-old dog with a circular mass on the ventral surface of the bladder that was diagnosed as a fibroma. The mass was removed surgically.

recommended because it causes unacceptable renal toxicity.[21,24] Cisplatin directly damages renal tubule cells, and piroxicam may interfere with renal blood flow.[24,27] Preliminary findings suggest that reducing the dosage of cisplatin in this combination does not prevent renal toxicity . In one study, a combination of carboplatin and piroxicam induced remission in 38% of dogs, but the duration of remission and survival were relatively short.[25] In another study, a mitoxantrone/piroxicam combination induced remission in 35% of dogs with minimal toxicity and resulted in a median survival time of 291 days.[26]

As a single agent, piroxicam is a useful palliative treatment for dogs with TCC.[1,2,23] The quality of life of dogs that receive piroxicam has been excellent. Responses in 62 dogs with TCC that received piroxicam as a single agent have included two patients with complete remission, nine with partial remission, 35 with stable disease, and 16 with progressive disease.[31] The two dogs that had a complete remission died of non-tumor-related causes more than 2 years after beginning piroxicam therapy and were free of tumor on postmortem examination. The survival time (median, 195 days) compared favorably to that of 55 dogs in the Purdue Comparative Oncology Program Tumor Registry that were treated with debulking surgery alone

TABLE 28–2	Results of Medical Therapy of Naturally Occurring Invasive Transitional Cell Carcinoma in Pet Dogs			
Drug	**N**	**% CR/PR***	**Survival (Days)**	**References**
Single-arm studies				
Cisplatin (60 mg/m²)	18	19	130	21
Cisplatin (50 mg/m²)	15	20	132	22
Piroxicam	62	18	195	1, 23
Cisplatin (60 mg/m²)/piroxicam	14	50	329	24
Carboplatin	12	0	132	10
Carboplatin/piroxicam	31	38	161	25
Mitoxantrone/piroxicam	55	35	291	26
Mitoxantrone	6	17	NA	2
Doxorubicin	5	20	NA	2
Actinomycin D	6	17	NA	2
Randomized trials				
Cisplatin (60 mg/m²)	8	0	300†	27
Cisplatin (60 mg/m²)/piroxicam‡	14	70	246	27

*CR, Complete remission (i.e., complete resolution of all clinical, radiographic, and ultrasonographic evidence of transitional cell carcinoma; PR, partial remission (i.e., 50% reduction or greater in tumor volume and no new tumor lesions); NA, not applicable.

†Dogs that initially received cisplatin alone and had tumor progression were then treated with piroxicam alone. Two dogs had partial remission, and five dogs had stable disease with piroxicam treatment. This may account for the favorable survival in that treatment arm.

‡Despite favorable survival data, the combination of cisplatin and piroxicam is considered significantly nephrotoxic and cannot be recommended.

(median survival, 109 days).[2] Piroxicam has been administered at a dosage of 0.3 mg/kg given PO once daily in dogs. Although most dogs tolerate the drug well, care must be taken to watch for gastrointestinal toxicity, particularly ulceration. If vomiting, melena, and anorexia occur, the drug must be withdrawn and supportive care provided as needed until the toxicity resolves. If piroxicam is to be reinstituted, concurrent administration of misoprostol reduces further gastrointestinal irritation from the piroxicam.

Specific protocols for the various chemotherapeutic agents are provided in Chapter 1.

Regardless of the treatment regimen pursued for TCC, basic concepts apply in tailoring therapy to the individual dog. The tumor or tumors should be staged completely and the extent of the disease and the size of lesions should be defined before and after 4 to 8 weeks of treatment. If the tumor is smaller or if it is stable in size (less than 50% change in tumor volume and no new tumor lesions) and if the treatment is acceptable with regard to any side effects, the current regimen may be continued. If complete remission occurs, treatment is continued for an additional 4 to 8 weeks in the hope of eradicating microscopic disease. Some oncologists recommend lifelong continuation of piroxicam, even with complete remission. Treatment is discontinued if progressive disease or unacceptable toxicity is noted.

Other therapies

Investigation of photodynamic therapy (PDT) as a treatment for TCC is underway.[31,33] PDT with 5-aminolevulinic acid (ALA) has potent effects in killing canine TCC cells *in vitro*.[32] In one study, ALA was given to healthy dogs, and induction of the photoactive metabolite, protoporphyrin IX (PpIX) was confined to the bladder mucosa.[33] ALA-based photodynamic therapy of TCC in five dogs resulted in tumor progression–free intervals ranging from 4 to 34 weeks (median, 6 weeks). ALA-based PDT was applied to a male dog with urethral TCC, and this dog was still disease free 1 year after treatment.[34]

Intravesical therapy, which frequently is used in noninvasive bladder cancer in humans, has not been studied to any extent in canine TCC. Human bladder tumors treated with this approach typically are superficial, noninvasive tumors that can be penetrated by intravesical drugs. Canine TCC is papillary and invasive into the lamina propria, and muscle layers may not be penetrated adequately by typical intravesical drugs.

Supportive care

Dogs with TCC are at high risk for secondary bacterial infections. Urinalysis and urine cultures should be performed regularly and antibiotics prescribed as needed. Urination should be monitored closely. If urinary tract obstruction occurs, catheterization, definitive anticancer therapy, antibiotics to reduce inflammation associated with secondary bacterial infection, or surgical debulking may be considered. As a palliative measure, prepubic catheters have been maintained for several months in dogs with bladder or urethral TCC.[16] Use of a low-profile gastrotomy tube as a prepubic cystotomy tube has also been described.[35]

Prognosis

Unfortunately, most dogs that develop TCC ultimately die of the disease. However, many dogs with TCC can live several months or longer with a good quality of life. Median survival times in several studies exceed 6 months.[31] Even with piroxicam treatment alone, approximately 20% of dogs survive longer than 1 year. Survival has been strongly associated with the TNM stage at the time of diagnosis (Table 28-3). According to data from 102 dogs with TCC at the PUVTH, factors associated with a more advanced TNM stage at diagnosis included a younger age (increased risk of nodal metastasis), prostate involvement (increased risk of distant metastasis), and a higher T stage (increased risk of nodal and distant metastasis).[2] A more advanced T stage at diagnosis and glandular differentiation in the histopathologic examination were associated with a poor response to medical therapy.

TABLE 28-3	**TNM Stage at Diagnosis and Survival of Dogs with Urinary Bladder Transitional Cell Carcinoma**		
Tumor Stage	Number of Dogs	Median Survival (Days)	Wilcoxon 2–Sample Test (p Value)
T_1 or T_2	82	218	0.0167
T_3	20	118	
N_0	86	234	0.0001
N_1	16	70	
M_0	99	203	0.0163
M_1	14	105	

Modified from Knapp DW, Glickman NW, DeNicola DB et al: Naturally-occurring canine transitional cell carcinoma of the urinary bladder: a relevant model of human invasive bladder cancer, Urol Oncol 5:47-59, 2000.

FELINE URINARY BLADDER TUMORS

Bladder cancer is rarely reported in cats. A series of 27 feline bladder tumors has been reported, which comprised 15 carcinomas, five benign mesenchymal tumors, five malignant mesenchymal tumors, and two cats with lymphoma.[36] Twenty of the cats were male and seven were female, and most were elderly.[36] Partial cystectomy was performed in nine cats, and four cats (two with leiomyoma, one with hemangiosarcoma, and one with leiomyosarcoma) survived longer than 6 months.[36]

URETHRAL TUMORS

Most urethral tumors are malignant epithelial tumors (TCC or squamous cell carcinoma); smooth muscle tumors are reported less frequently.[37] The practitioner must be especially careful to distinguish urethral tumors from granulomatous urethritis.[38] Multiple chondrosarcomas in the urethra of a dog, which had a long-term survival after surgery, has been reported.[39] Most urethral tumors are not resectable. Urinary diversion techniques have been described, including vaginourethroplasty, urethral reconstruction, and the use of urethral stents.[40-42] The response of urethral TCC to chemotherapy or piroxicam appears similar to that of TCC of the urinary bladder.[21,23,27]

RENAL TUMORS

Primary renal tumors are uncommon in dogs, accounting for fewer than 2% of all canine cancer; whereas tumors that have metastasized to the kidneys are more common.[43] Most primary renal tumors are malignant, and more than half of these are epithelial in origin. A report of 54 primary renal tumors in dogs included 35 renal tubular cell carcinomas (RCCs), five TCCs, three renal transitional cell papillomas, and smaller numbers of anaplastic carcinomas, anaplastic sarcomas, fibromas, hemangiosarcomas, lymphomas, and nephroblastomas.[43] Lymphoma and RCC often are bilateral. RCC can be highly invasive into adjacent structures and may invade the vena cava. Approximately 10% of primary renal tumors derive from embryonal pluripotential blastema (Wilms' tumor, nephroblastoma, embryonal nephroma).[44] An unusual syndrome reported in German shepherds consists of dermal fibrosis, concomitant renal cystadenocarcinoma, and uterine tumors in affected females (Figure 28-5).[45,46] Lymphoma is the most common renal neoplasm of cats, and other tumors occur less frequently.[47]

A

B
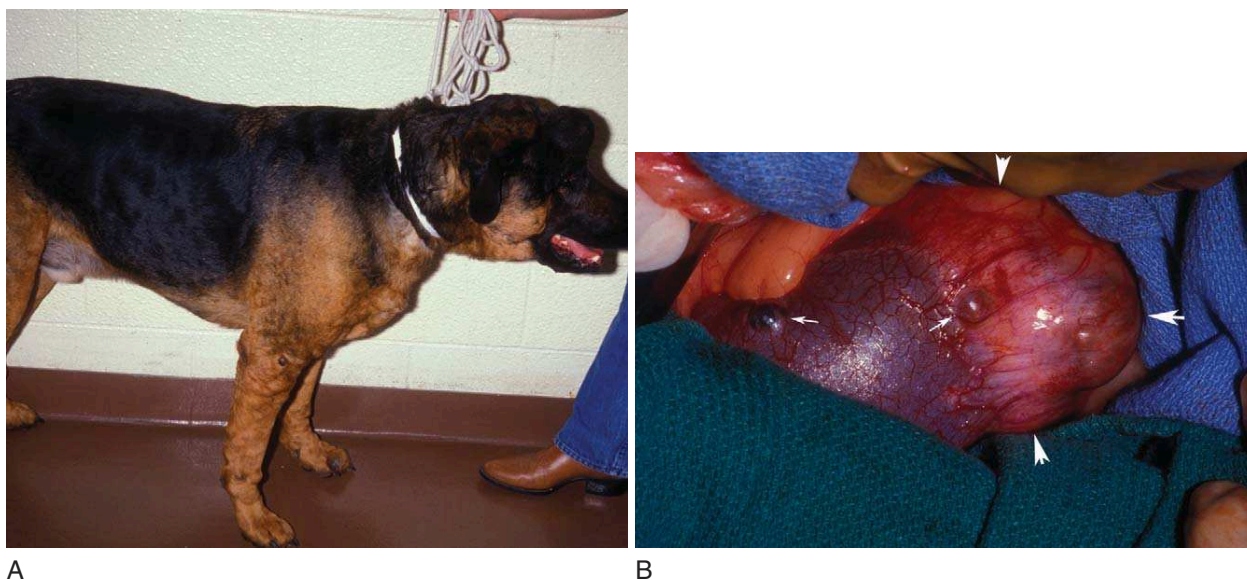
Figure 28-5

A middle-aged German shepherd with numerous firm, fibrous, painless nodules in the skin and subcutaneous tissues **(A).** Note the large mass over the frontal sinus area. In the operative view of the kidney **(B),** note the several small cystic masses *(small arrows)* and a larger mass *(large arrow)* on the right pole of the kidney. The diagnosis was bilateral cystadenocarcinoma. No treatment was performed. The dog survived for 18 months after the skin and subcutaneous masses were detected.

Diagnosis and Clinical Staging

Most renal tumors are found in older dogs. Nephroblastoma may occur in young, middle-aged, or old animals. The male to female ratio of epithelial renal tumors has been reported as 1.6:1.[43] Clinical signs in dogs with renal tumors often are nonspecific and may include anorexia, depression, weight loss and, in some cases, abdominal distention. Pain as a result of bone metastases or hypertrophic osteopathy is uncommon. The physical examination may reveal a mass or, in some cases, pain in the region of the kidneys. Gross hematuria is not a consistent finding. German shepherds with slow-growing, firm skin and subcutaneous fibrous masses should be evaluated for possible concomitant renal cystadenocarcinoma.[45]

Laboratory findings in dogs with renal tumors may include mild to moderate anemia secondary to hematuria. Polycythemia, possibly as a result of increased production of erythropoietin, has been reported in a small number of dogs and in one cat.[48-50] Marked neutrophilia has been reported with canine RCC.[51,52] Leukocytosis and hypertrophic osteopathy were reported in a dog with renal TCC, and the paraneoplastic lesions resolved with surgical removal of the tumor.[53] The results of a serum biochemical profile in dogs with renal tumors may be normal or may reveal azotemia.

Histopathologic examination of tumor tissue is required for diagnosis. Tumor tissue can be obtained by transabdominal biopsy or at surgery. Ultrasound-guided biopsies may be more accurate and safer for obtaining tissue transabdominally. Clinical staging should include radiography of the thorax and abdomen (Figure 28-6) and abdominal ultrasonography. Excretory urography and computed tomography (CT) also may provide useful information, especially for surgical planning. Evaluation of possible extension of the tumor into the vena cava is important if surgery is contemplated. If nephrectomy is considered as a treatment option, determination of the glomerular filtration rate (GFR), ideally through nuclear scintigraphy, should be considered.

Treatment and Prognosis

Nephrectomy is the treatment of choice for unilateral tumors that have not metastasized. Surgery should include removal of the ureter and possibly retroperitoneal muscle and tissue if the tumor has extended through the capsule and invaded surrounding tissues. In a series of nine dogs with RCC that survived at least 22 days after nephrectomy, the median survival time was 8 months.[43] The survival times of three dogs with TCC of the kidney that had a nephrectomy were 3, 5, and 25+ months.[43]

Lymphoma of the kidneys generally is treated with chemotherapy (see treatment of multicentric lymphoma in Chapter 31).[47] Successful chemotherapy protocols for other forms of renal cancer have not been identified.

The prognosis for renal tumors has not been clearly defined. Only a limited number of cases of renal tumor have been reported in the veterinary literature. Metastasis is a concern for any of the malignant renal tumors. Pulmonary metastasis was detected radiographically in 13 of 38 dogs with primary renal tumors.[43]

A   B
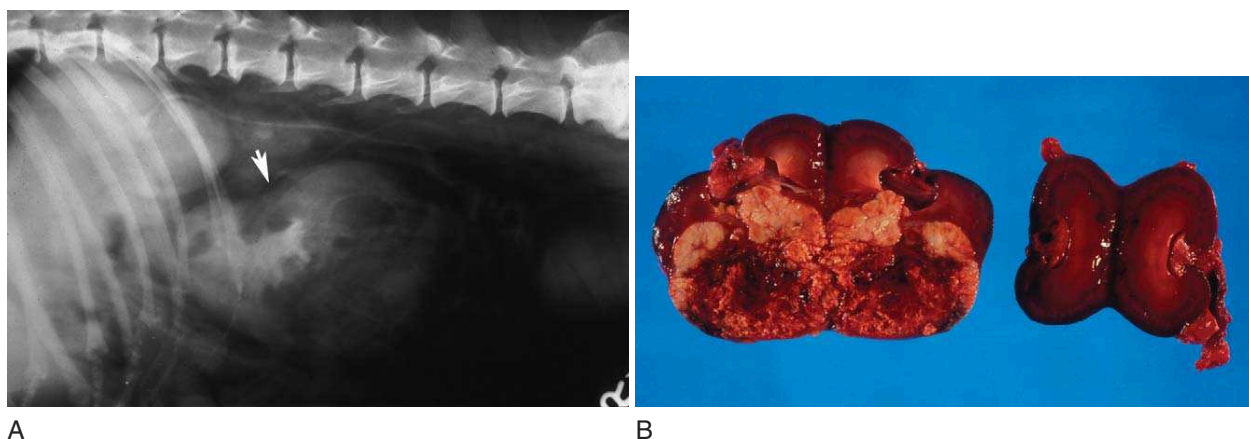
Figure 28-6

A, Lateral abdominal radiograph of a dog during intravenous pyelography. Note the irregular filling of the caudal pole of the left kidney *(arrow)*. The diagnosis was renal adenocarcinoma. Metastases were noted in the lung and bones, and no treatment was pursued. **B,** Gross abnormalities were noted in the kidneys during a postmortem examination.

Although the survival time for dogs with malignant renal tumors generally is short, long-term survival (up to 4 years) has been reported.[43,48,54,55] Nephroblastoma is less metastatic, and nephrectomy may be curative in some cases. Benign renal tumors occur infrequently and in some cases appear cured with surgery.[56]

COMPARATIVE ASPECTS

Urinary bladder cancer is newly diagnosed in more than 54,000 humans each year in the United States.[51] Cigarette smoking is a major cause of human bladder cancer. In humans, approximately 80% of bladder tumors are superficial, low-grade tumors in the bladder mucosa.[57] These tumors generally respond well to transurethral resection and intravesical therapy, although progression to invasive TCC is a risk. Approximately 20% of human bladder cancers are higher grade invasive carcinomas at the time of diagnosis. Metastasis to the regional lymph nodes, lungs, and other organs occurs in approximately 50% of cases of invasive TCC.[57] More than 14,000 people die of bladder cancer each year in the United States, and most deaths are caused by metastatic, chemoresistant TCC.[57] Invasive TCC in humans is very similar to canine TCC in histopathologic characteristics, biologic behavior, and response to therapy.[2] Successful therapies in dogs are being adapted for use in human clinical trials.

Important types of renal cancer in humans include RCC, TCC of the renal pelvis, and Wilms' tumor (nephroblastoma).[57] RCC is newly diagnosed in 28,000 people and results in 12,000 deaths each year in the United States.[57] Risk factors include cigarette smoking, asbestos exposure, and work in the leather industry. Human RCC arises from the epithelial cells of the proximal renal tubules. Approximately 4% of RCCs are hereditary, and the remaining tumors are considered sporadic. At least three different types of hereditary RCC exist. Metastasis is present at diagnosis in 30% of patients with RCC. Common sites of metastasis include the lungs, lymph nodes, bone, liver, and other soft tissues. RCC has been treated with surgery, radiation, chemotherapy, and biologic therapy (interferon and interleukin-2).[57] Canine RCC has not been studied with the scrutiny necessary to determine whether it mimics human RCC.

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CHAPTER **29**

Tumors of the Nervous System

Richard A. LeCouteur, Stephen J. Withrow

INTRACRANIAL NEOPLASIA

Incidence and Risk Factors

Dogs

Few data exist concerning the incidence of brain tumors in dogs, although intracranial neoplasms appear to be more common in dogs than in other domestic species.[1,2] An incidence rate of brain tumors in dogs of 14.5 per 100,000 of the population at risk has been reported.[3] The most common sites for neoplasia to occur in immature dogs, in decreasing order, are the hematopoietic system, brain, and skin.[4-8]

A broad spectrum of tumor types occur in dogs.[2,9,10] Gliomas (e.g., astrocytomas and oligodendrogliomas) and meningiomas are the most commonly recognized intracranial neoplasms of dogs.[1,11,12] Canine primary brain tumors usually are solitary; however, multiple primary brain tumors have been reported. Multiple meningiomas,[13,14] and cerebrospinal fluid (CSF) metastases of medulloblastoma[10] or choroid plexus carcinoma[15] occur in dogs. Multiple tumors of different histologic type may occur rarely. Extracranial metastases of primary brain meningiomas have been reported.[16]

The most frequently seen secondary tumors of dogs include local extension of nasal adenocarcinoma;[17] metastases from mammary, prostatic, or pulmonary adenocarcinoma; metastases from hemangiosarcoma; and extension of pituitary adenoma or carcinoma.[9,10,13,17,18] Nerve sheath tumors arising from cranial nerves (particularly the oculomotor nerve and the trigeminal nerve) may occur in dogs.[19] Skull tumors that affect the brain by local extension include osteosarcoma, chondrosarcoma, and multilobular osteochondrosarcoma.[20,21]

Brain tumors occur in dogs of any age, all breeds, and of either sex, and they are most frequent in older dogs, with the greatest incidence in dogs over 5 years of age.[1,10,22,23] Certain breeds have a higher incidence of some tumor types. Glial cell tumors and pituitary tumors occur commonly in brachycephalic breeds, whereas meningiomas occur most frequently in dolichocephalic breeds.[9] Canine breeds that are over-represented include the boxer, golden retriever, Doberman pinscher, Scottish terrier, and Old English sheepdog.[22]

Cats

Brain tumors occur less commonly in cats than in dogs. An incidence of approximately 3.5 per 100,000 population has been reported.[3,24] There does not appear to be a breed predisposition for the development of intracranial tumors in cats. Meningioma is the most common primary brain tumor of cats,[1,25-28] and older male cats appear to be most susceptible to meningiomas. Meningiomas involving multiple intracranial sites (including the third ventricle) occur relatively commonly in cats.[1,29-31] An unusually high incidence of meningiomas has been reported in cats with mucopolysaccharidosis type I.[32]

Primary brain tumors other than meningiomas may occur in cats.[3,29] Tumors that have been reported include astrocytoma, ependymoma, oligodendroglioma, choroid plexus papilloma, medulloblastoma, plasma cell tumor, lymphoma, olfactory neuroblastoma, and gangliocytoma.[1,29,30,33] Lymphoma of the brain may be primary or secondary, or it may be an aspect of multicentric lymphoma of cats.

Secondary tumors that have been reported to occur in the brain of cats include pituitary macroadenomas and macrocarcinomas, and metastatic carcinomas. Local extension may occur either from tumors of the middle ear cavity (e.g., squamous cell carcinoma), nasal cavity (e.g., nasal adenocarcinoma), or the skull (e.g., osteosarcoma).

The cause of brain tumors in dogs and cats is not known. Dietary, environmental, genetic, chemical, viral, traumatic, and immunologic factors may be considered.[34] Although the significance of a high incidence of meningiomas in the brain of young cats affected by mucopolysaccharidosis type I is unknown, genetic factors may play a role in tumor development (e.g., a specific chromosomal deletion).[32,34]

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Pathology

Intracranial neoplasms of cats or dogs may be classified as either primary or secondary, depending on the cell type of origin.[1,9,35,36] Primary brain tumors originate from cells normally found within the brain and meninges, including the neuroepithelium, lymphoid tissues, germ cells, endothelial cells, and malformed tissues. Secondary tumors are either neoplasms that have reached the brain by hematogenous metastasis from a primary tumor outside the nervous system, or neoplasms that affect the brain by local invasion, or extension, from adjacent non-neural tissues such as bone.[2,18] Pituitary gland neoplasms (adenomas or carcinomas) and tumors arising from cranial nerves (e.g., nerve sheath tumor of the trigeminal, oculomotor, or vestibulocochlear nerves) are considered secondary brain tumors, as they affect the brain by means of local extension.

Tumors of the central nervous system (CNS) present a variety of histologic appearances and cytologic derivations, reflecting the morphologic complexity of the brain and spinal cord.[34,37-40] The classification of CNS tumors in dogs and cats is primarily based on the characteristics of their constituent cell type, pathologic behavior, topographic pattern, and secondary changes present within and surrounding the tumor.[1,2,9,35,41-44] A classification system based on the World Health Organization classification of human brain tumors published in 1993 provides a basis for the classification of primary brain tumors of animals (Box 29-1).[45]

Glial neoplasms are a pleomorphic group, and diverse classification schemes have been devised to categorize them. Classification of the glial subset of neuroepithelial tumors is based on the predominant cell type (e.g., astrocyte or oligodendrocyte).[45] Since the mid-1990s, pathologists have increasingly recognized that in addition to monotypic gliomas, there are neoplasms that exhibit a blend of two or more neoplastic glial cell types. Many pathologists tend to regard these tumors as arising by "dedifferentiation" of differentiated cell types and to classify them using the most morphologically recognizable cell and tissue pattern.[46] Admixture of neoplastic cells occurs frequently in these tumors and should not, unless substantial, lead to an excessive diagnosis of oligoastrocytoma (see Box 29-1), which is the equivalent of the previous designation in veterinary medicine of "mixed glioma."[45]

Embryonal tumors have been consolidated under the single term *primitive neuroectodermal tumors* (or *PNETs*) to accommodate their anaplastic nature.[47] This classification is based on the concept that all embryonal tumors are derived from a germinal neuroepithelial cell that has a potential to differentiate along a number of neuroectodermal cell lines, primarily neuronal, ependymal, and glial. All tumors within the group of embryonal tumors are biologically malignant.[34] PNET is a generic term for neoplasms that are morphologically indistinguishable from the cerebellar medulloblastoma but are located at other sites in the CNS (see Box 29-1).[34,45] PNETs that may be specifically identified histogenetically

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| **Box 29-1** | **Histologic Classification of Primary Tumors of the Central Nervous System of Dogs and Cats** |

I. Glial Neoplasms
   A. Astrocytoma
   B. Oligodendroglioma
   C. Oligoastrocytoma
II. Tumors of the Ependyma and Choroid Plexus
   A. Ependymoma
   B. Choroid plexus papilloma
   C. Choroid plexus carcinoma
III. Neuronal and Mixed Neuronal-Glial Neoplasms
   A. Gangliocytoma
   B. Ganglioglioma
IV. Embryonal Tumors
   A. Olfactory neuroblastoma (esthesioneuroblastoma)
   B. Medulloblastoma
   C. Neuroblastoma
   D. Other primitive neuroectodermal tumors (PNETs)
   E. Intradural-extramedullary spinal cord tumor of young dogs

V. Tumors of the Meninges
   A. Meningioma
VI. Lymphomas and Other Hematopoietic Tumors
   A. T- and B-cell lymphomas of the CNS
   B. Histiocytic sarcoma
   C. Neoplastic reticulosis
VII. Rare Primary CNS Tumors, Tumorlike Lesions, Hamartomas, and Cysts
   A. Pineal tumors
   B. Germ cell tumors
     i. Germinoma
     ii. Teratoma
   C. Primary melanoma of the CNS
   D. Chordoma
   E. Hamartoma
   F. Cysts
     i. Epidermoid
     ii. Dermoid
   G. Granular cell tumor

are designated as a separate subgroup (e.g., cerebellar medulloblastoma).

Tumors of the CNS arising from lymphoreticular cells traditionally have been grouped under a heading of reticulosis or histiocytic lymphoma.[48-51]Classification of CNS neoplasms within the reticulosis complex originally described for cats and dogs is confusing.[41,50,52,53] These single or multiple mass lesions are characterized histologically by a perivascular proliferation of "reticulohistiocytic cells," with different patterns of reticulin production, admixed with varying populations of inflammatory cells.[50] This morphologically heterogeneous group has included descriptions of types of both inflammatory and neoplastic reticulosis and of microgliomatosis. Currently, the inflammatory subtype in dogs is considered to be part of the spectrum of canine granulomatous meningoencephalomyelitis (GME).[54-56] Based on immunocytochemical staining, neoplastic reticulosis may comprise a neoplastic or immunoproliferative accumulation of cells expressing various macrophage or histiocytic-like cell markers.[57]

T and B cell lymphoma as well as histiocytic sarcoma fall within this category (see Box 29-1).[13,58-60] Microgliomatosis is a questionable entity that was originally proposed as a neoplastic proliferation of endogenous microglial cells and was thus classified within the reticulosis complex.[9] Current findings suggest that there is insufficient evidence to separate microgliomatosis from either astrocytoma or primitive neuroectodermal tumor.[9,61-63]

The terms *benign* and *malignant* should be used with care when applied to brain neoplasms. In assessing the malignant potential of a brain tumor, the difference between cytologic and biologic malignancy should be emphasized. Cytologic malignancy is a morphologic assessment of anaplasia, based on cytologic and nuclear pleomorphism, cellularity, necrosis, mitoses, and invasiveness. Biologic malignancy is the likelihood that a tumor will kill the animal. Most cytologically malignant brain tumors are also biologically malignant, despite the treatments presently available. Cytologically benign tumors of the brain may also be biologically malignant because of various secondary effects such as increased intracranial pressure (ICP). Particular care must be exercised in the use of the term *benign* when discussing the meningiomas of dogs and cats. Canine meningioma is characterized as "benign," yet it locally infiltrates along the Virchow-Robin space and invariably lacks demarcation from normal brain tissue.[64] In cats, meningiomas are almost always well defined and have a clear demarcation between the tumor and normal brain.[64] The growth rate of meningiomas in cats appears to be slow when compared to that of canine meningiomas.

Brain tumors result in cerebral dysfunction by causing both primary effects, such as infiltration of nervous tissue or compression of adjacent anatomic structures, and secondary effects, such as hydrocephalus. Additional primary effects include disruption of cerebral circulation, or local necrosis, which may result in further damage to neural tissue. The most important secondary effects of a primary brain tumor include disturbance of CSF flow dynamics, elevated ICP, cerebral edema, or brain herniation.[65] Secondary effects usually are more diffuse or generalized in their clinical manifestations and may "mask" the precise location of a focal intracranial lesion.

Primary brain tumors often are slow growing; however, because the brain is contained within the confines of the calvaria, even a slow-growing tumor may have devastating effects. In slow-growing lesions of the brain, gradual compression permits surrounding structures to adapt to increasing pressure. During the time the brain is able to compensate, there may be a prolonged history of vague signs (e.g., subtle behavior alterations). However, even with a very slowly progressive tumor, clinical signs may progress rapidly when compensatory mechanisms have been exhausted. Rapidly growing tumors may not permit the process of compensation to occur to the same degree as with a slow-growing tumor, and in such cases a sudden onset of catastrophic neurologic dysfunction may occur in the absence of premonitory signs. Should a neoplasm erode or obstruct a major blood vessel, causing hemorrhage or infarction, an acute onset of neurologic deficits may ensue. Secondary brain tumors, particularly metastases, often demonstrate an acute progression of signs.

An aspect of the pathogenesis of brain tumors that is of practical concern is the method by which a brain tumor may spread. The patterns of spread of brain tumors are quite distinct from those of other tumors because of several factors, including lack of a well-developed lymphatic system within the brain. The major patterns of spread involve local invasion and CSF seeding. Brain tumors, particularly astrocytomas, contain cells that are capable of invading the normal brain to a remarkable degree. Seeding may occur by spread along the surface of the brain to local sites or by so-called drop metastases, which spread by way of CSF to the spinal subarachnoid space and then form secondary tumors (Figure 29-1). Systemic spread by means of the hematogenous route may also occur, though extracranial metastasis rarely is seen.[16,29] As the treatment of primary brain tumors becomes more widespread, the subject of tumor spread will become more important. It is likely that an increase in various patterns of spread will be evident in association with the increased life expectancy that accompanies the successful therapy of dogs or cats with a primary brain tumor.

History and Clinical Signs

History and neurologic examination are the first steps to a definitive diagnosis in the evaluation of a dog or cat
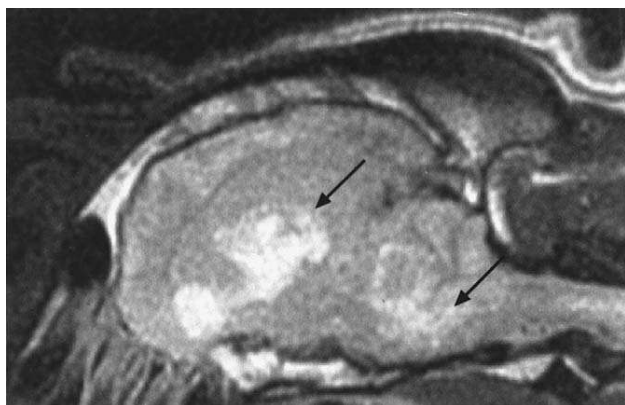
Figure 29-1

Parasagittal T1-weighted MR image of the brain of a 6-year-old castrated male English bulldog after the intravenous administration of contrast medium. Note the irregularly contrast-enhancing oligodendroglioma in the frontal lobe of the cerebrum extending into the floor of the right lateral ventricle *(top arrow)* and metastasis of this mass to the fourth ventricle via the cerebrospinal fluid *(bottom arrow)*.

suspected of having a brain tumor. The nature and course of neurologic signs resulting from a brain tumor depend primarily on the location, extent, and rate of growth of the neoplasm.[1,23,66]

Many dogs or cats with a brain tumor will have a long history of vague signs (e.g., behavior alterations) that often are overlooked by an owner or veterinarian until the signs of brain dysfunction are well developed. These signs include subtle behavior alterations that may progress slowly over many months. While the exact cause of these vague signs may never be understood, it is interesting to compare this situation with experience in humans. In the majority of humans with a brain tumor, the initial symptom is headache, which is often persistent, severe, and may be worse in the morning. Because headache is a verbalized phenomenon in humans, it is impossible to recognize this sign of dysfunction with certainty in dogs or cats. However, cats and dogs may exhibit abnormal behavior that is consistent with the presence of a headache, such as not wishing to be handled or hiding during the day. In a series of cats with meningiomas examined by the author, the owners noted subtle alterations in behavior, such as decreased frequency of purring or diminished activity levels, for more than a year before the onset of focal neurologic signs.

The most frequently recognized clinical sign associated with a brain neoplasm of a dog or cat is seizures, particularly if the first seizure occurs after the animal is 4 years old.[23,67] This may take the form of a generalized seizure, or it may be a focal seizure. Focal seizures may

aid in localizing a neoplasm. In a large compilation of feline intracranial neoplasia, the most common neurologic signs were altered consciousness, circling, and seizures.[24] Other clinical signs frequently associated with a brain tumor in dogs and cats include circling, altered posture, gait abnormalities, ataxia, head tilt, behavior change, depression, incontinence and cervical spinal hyperesthesia.[23,24,67-69]

Focal neurologic signs usually occur in association with the primary effects of a fairly well developed mass lesion. Should a neoplasm involve the brain stem, cranial nerve deficits may be recognized. Weakness, sensory loss, or deficits in vision, hearing, or smell may occur in association with tumors at specific sites.[1] Weakness and sensory abnormalities usually denote a lesion in the cerebral frontoparietal sensorimotor regions or their deeper pathways. Visual deficits involve the visual pathways from the optic nerve to the occipital lobe of the cerebrum.[70] Hearing loss involves the cerebellomedullary region, the brain stem, or temporal lobes of the cerebrum. Problems with the ability to smell are seen in association with lesions of the cribriform plate, olfactory bulb and peduncle, and pyriform or temporal lobes of the cerebrum, or other rhinencephalic connections. Difficulties with balance or gait suggest cerebellar or vestibular involvement.

The secondary effects of brain tumors, such as increased ICP, represent further advancement of tumor growth.[66] By the time these effects occur, either a large tumor or significant cerebral edema is present. The signs include alterations in behavior (e.g., lethargy, irritability), circling, head pressing, compulsive walking, altered states of consciousness, or associated locomotor disturbances.[23] The majority of cats or dogs with a brain tumor will be presented to a veterinarian with problems that relate to the secondary effects of a tumor. This in turn suggests that brain tumors of cats and dogs usually have reached a large size by the time an owner seeks veterinary attention for a pet. This is especially true with frontal lobe neoplasms of dogs, which may reach a very large size before causing clinical signs.[71,72] In one study of 43 dogs with brain tumors affecting the rostral cerebrum, those animals with seizures or behavior abnormalities, or both, developed demonstrable neurologic deficits within 3 months of onset of these signs.[71] In the same study, the clinical course from detection of demonstrable neurologic deficits to death (or euthanasia) was less than 2 weeks in the absence of therapeutic intervention.

Diagnostic Techniques and Workup

On the basis of signalment, history, and results of complete physical and neurologic examinations, it is possible to localize a problem to the brain and, in some cases, to determine the approximate location.

However, the signs that result from a disease in a given location in the nervous system will be similar, regardless of the precise cause.[73] The categories of disease that may result in clinical signs similar to those of a brain tumor include congenital disorders, infections, immunologic and metabolic disorders, toxicities, nutritional disorders, trauma, vascular disorders, degeneration, and idiopathic disorders. All of these other categories of disease must be eliminated before a diagnosis of brain tumor may be made. For this reason, it is essential to follow a logical diagnostic plan for a dog or cat that has signs of brain dysfunction.

Minimum database

A minimum database for a dog or cat with clinical signs of brain dysfunction should include a hemogram, serum chemistry panel, and urinalysis. Survey radiographs of the thorax and abdominal ultrasound examination help to rule out a primary malignancy elsewhere in the body. The major objective in completing these tests is to eliminate extracranial causes for signs of cerebral dysfunction.

Radiography

Plain skull radiographs are of limited value in the diagnosis of a primary brain tumor; however, they may be helpful in detecting neoplasms of the skull or nasal cavity that involve the brain by local extension. Occasionally, lysis or hyperostosis of the skull may accompany a primary brain tumor (e.g., meningioma of cats), or there may be radiographically visible mineralization within a neoplasm (Figure 29-2).[1,74] General anesthesia is required for precise positioning of the skull for radiographs, and various projections have been recommended to identify abnormalities.[1]

Cerebrospinal fluid analysis

Analysis of CSF is recommended as an aid in the diagnosis of a brain tumor.[75-77] The results of CSF analysis may help to rule out inflammatory causes of cerebral dysfunction, and in some cases they may support a diagnosis of a brain tumor. Care should be used in the collection of CSF, because frequently an increased ICP may be present in association with a brain tumor, and pressure alterations associated with CSF drainage may lead to brain herniation.[65] CSF collection usually is delayed until advanced imaging has been completed in order to evaluate factors such as the presence of cerebral edema or hemorrhage. Hyperventilating the patient and administering mannitol may help to decrease elevated ICP prior to CSF collection.

In general, increased CSF protein content and a normal to increased CSF white blood cell count have been considered typical of a brain neoplasm.[78-82] In one study, only 39.6% of dogs with a primary brain tumor exhibited typical CSF alterations.[78] The results of CSF

analysis were normal in 10% of the dogs in this study, while the remaining 50.4% of dogs had a variety of nonspecific CSF changes. The CSF from dogs with a meningioma often may have an elevated white blood cell count (>50/μl), with more than 50% of these cells being polymorphonuclear leukocytes. In another study, glial cell tumors predominated among those that resulted in CNS inflammation.[71] Neoplastic cells may be present in CSF,[83] particularly when sedimentation techniques are used for analysis.[22,79,80] The use of CSF protein electrophoresis,[84] and IgG index of CSF,[85] may aid in the determination of the presence of a brain neoplasm. It appears that CSF alterations seen in association with feline brain tumors are similar to those described for dogs.[86]

Advanced imaging

Numerous brain imaging techniques such as ventriculography, cerebral angiography, cavernous sinus venography, cisternography,[87,88] and scintigraphy[89,90] have been used in dogs and cats.[17,91] However, each of these techniques has severe limitations in that they fail, in most instances, to define the exact extent of a neoplasm and its precise relationship to surrounding structures. This information is essential for establishing an accurate treatment plan for a cat or dog with a cerebral neoplasm.

Consequently, despite the availability of these techniques, significant advances in therapy for intracranial neoplasms did not occur until imaging techniques were developed that provided precise information regarding location and extent of a neoplasm. With the availability of computed tomography (CT), a basis for the development of therapeutic plans for dogs and cats with a brain tumor was available.[1,15,69,92-99] CT accurately determines the presence, location, size, and anatomic relationships of many intracranial neoplasms. More recently, magnetic resonance imaging (MRI) has allowed these principles to be advanced even further.[1,97-105] Images obtained by means of MRI are superior to those of CT in certain brain regions (e.g., the brain stem) (Figure 29-3).[106,107]

CT and MRI should be seen as complementary diagnostic imaging modalities, each with its own advantages and disadvantages.[106] CT excels in the detection of osseous changes, such as skull fractures or sclerotic changes in the tympanic bullae. Certain intracranial mass lesions may not be visible by means of CT, probably as a result of diffuse distribution, similar attenuation to surrounding normal tissue, and minimal or absent contrast enhancement. It is now generally accepted that MRI is superior to CT in the detection of many of the features associated with brain tumors, such as edema, cyst formation, changes in vascularity, hemorrhage, and necrosis, and that MRI provides superior soft tissue detail when compared to CT.[83,107] MRI permits visualization of subtle changes accompanying nonenhancing or diffuse brain lesions not easily seen
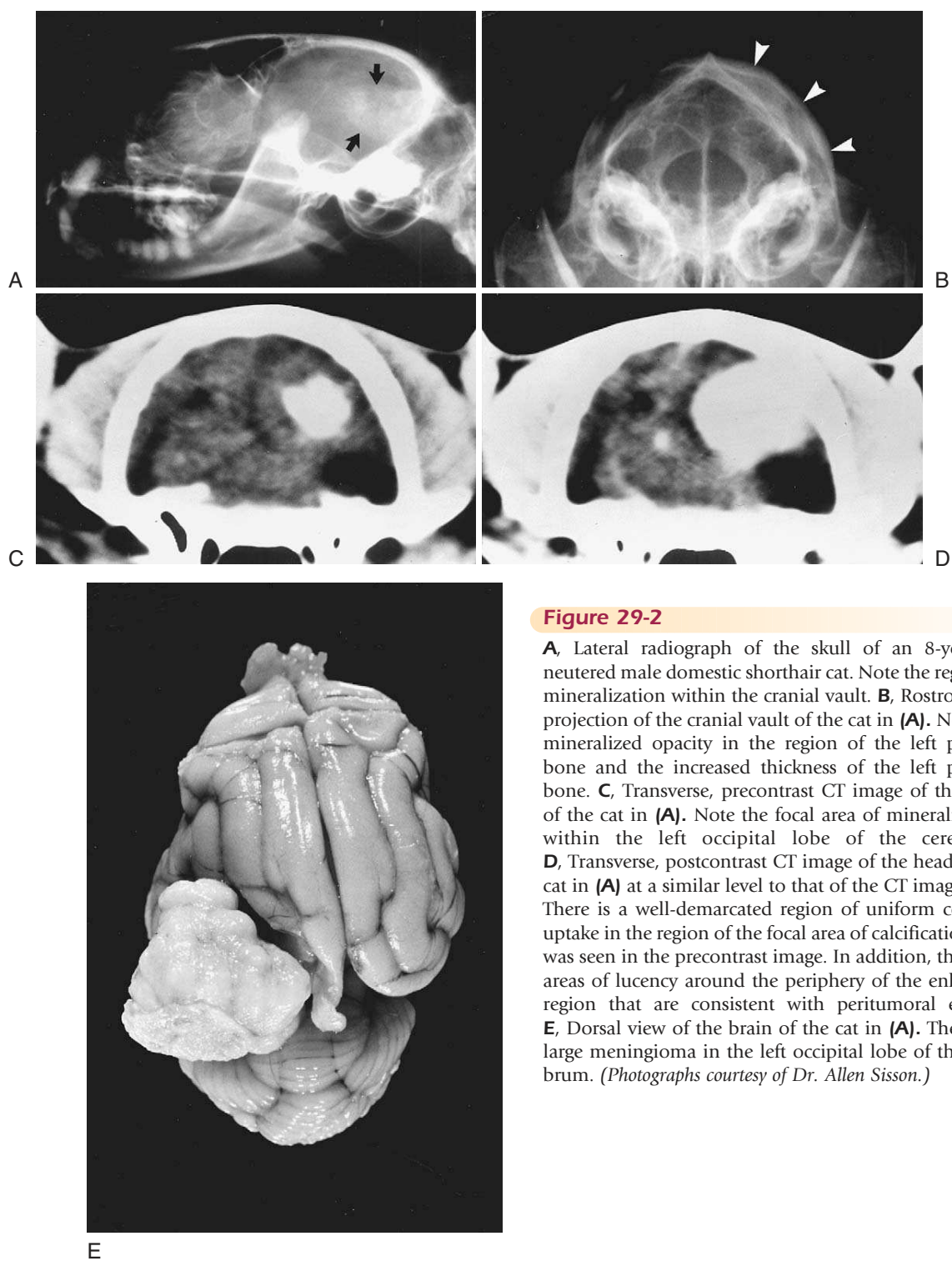
Figure 29-2

A, Lateral radiograph of the skull of an 8-year-old neutered male domestic shorthair cat. Note the region of mineralization within the cranial vault. **B**, Rostrocaudal projection of the cranial vault of the cat in **(A).** Note the mineralized opacity in the region of the left parietal bone and the increased thickness of the left parietal bone. **C**, Transverse, precontrast CT image of the head of the cat in **(A).** Note the focal area of mineralization within the left occipital lobe of the cerebrum. **D**, Transverse, postcontrast CT image of the head of the cat in **(A)** at a similar level to that of the CT image in C. There is a well-demarcated region of uniform contrast uptake in the region of the focal area of calcification that was seen in the precontrast image. In addition, there are areas of lucency around the periphery of the enhanced region that are consistent with peritumoral edema. **E**, Dorsal view of the brain of the cat in **(A).** There is a large meningioma in the left occipital lobe of the cerebrum. *(Photographs courtesy of Dr. Allen Sisson.)*

B

C
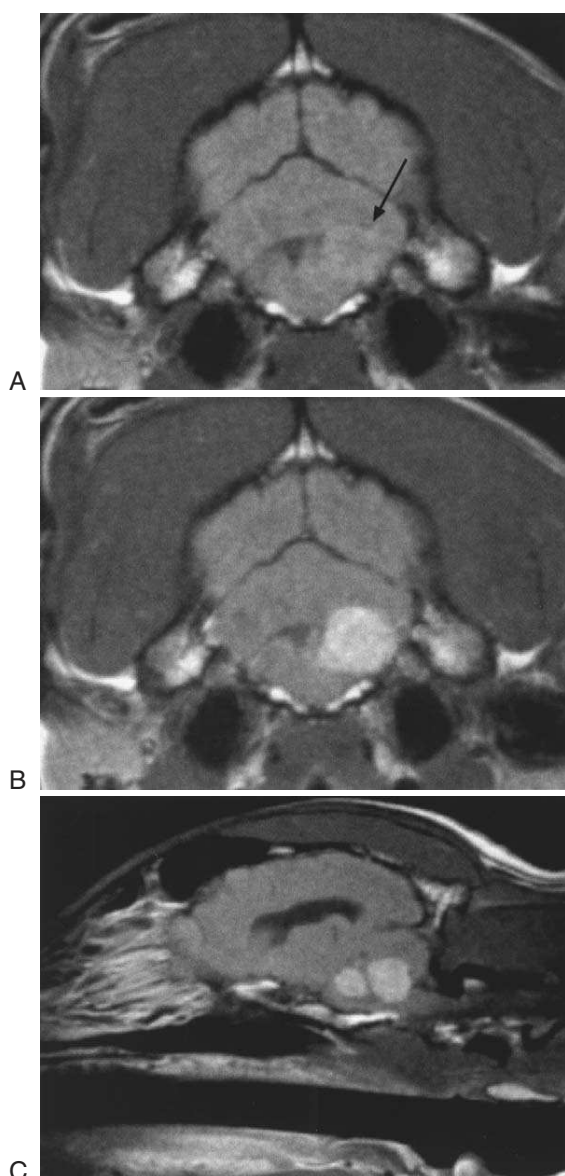
Figure 29-3

Transverse precontrast **(A)**, transverse postcontrast **(B)**, and parasagittal postcontrast **(C)** T1-weighted MR images of the brain of a 9-year-old spayed female Alaskan Malamute. On precontrast T1-weighted images a well-circumscribed, slightly hyperintense mass is evident in the left cerebellomedullary angle. The mass is uniformly contrast enhancing **(C).** On the parasagittal image **(C)**, the mass is apparently causing an obstructive hydrocephalus, as evidenced by ventriculomegaly. The dog survived for 14 months following surgical removal of the mass (choroid plexus papilloma) and radiation therapy.

using CT (Figure 29-4). The multiplanar imaging capability of MRI also permits better definition of the anatomic relationships of a tumor and surrounding normal structures. The high-resolving capabilities and improved contrast available with MRI makes this form of imaging more sensitive, and therefore preferable to CT, in the diagnosis of the majority of primary brain tumors.

While the major tumor types in dogs have been reported to have characteristic CT or MRI features,[94,97] remember that non-neoplastic space-occupying lesions may mimic the CT or MRI appearance of a neoplasm[93] and occasionally a metastasis may resemble a primary brain tumor on CT or MR images (Figure 29-5). In addition there is sufficient overlap in the appearance of different types of primary brain tumors to preclude making a definite diagnosis on the basis of imaging alone.[106,108]

Biopsy

Biopsy remains the sole method available for the definitive antemortem diagnosis of brain tumor type in cats or dogs[106,109-111] and is an essential step before considering any type of therapy; however, biopsy is not always attempted because of practical considerations, such as cost and morbidity.[111] Several biopsy methods have been described, including ultrasound-guided biopsy,[112] freehand, and stereotactic CT-guided biopsy.[111,113] The most recent advance in the biopsy of brain tumors of dogs and cats has been the modification of a CT-guided stereotactic brain biopsy system for use in cats and dogs.[102,114,115] This CT-guided stereotactic biopsy system provides a relatively rapid and extremely accurate means of tumor biopsy, with a low rate of complications (Figure 29-6). Cytologic evaluation of brain tumor smear preparations, rapidly fixed in 95% alcohol and stained with hematoxylin and eosin, may be done within minutes of biopsy collection.[107] Diagnostically accurate information from this rapid technique is generally available from both primary and metastatic nervous system tumors. Recently, fine-needle aspiration (FNA) cytology has been compared to tissue biopsy for the diagnosis of canine brain tumors, and while FNA was sensitive, it was not as definitive as biopsy in determining specific type of cerebral neoplasm.[109]

Therapy

The major goals of therapy for a brain tumor are to control secondary effects, such as increased ICP or cerebral edema,[17] and to eradicate the tumor or reduce its size.[91] Beyond general efforts to maintain homeostasis, palliative therapy for dogs or cats with a brain tumor consists of glucocorticoids for edema reduction and, in some cases (e.g., lymphoma), for retardation of tumor growth.[116] Some animals with a brain tumor will
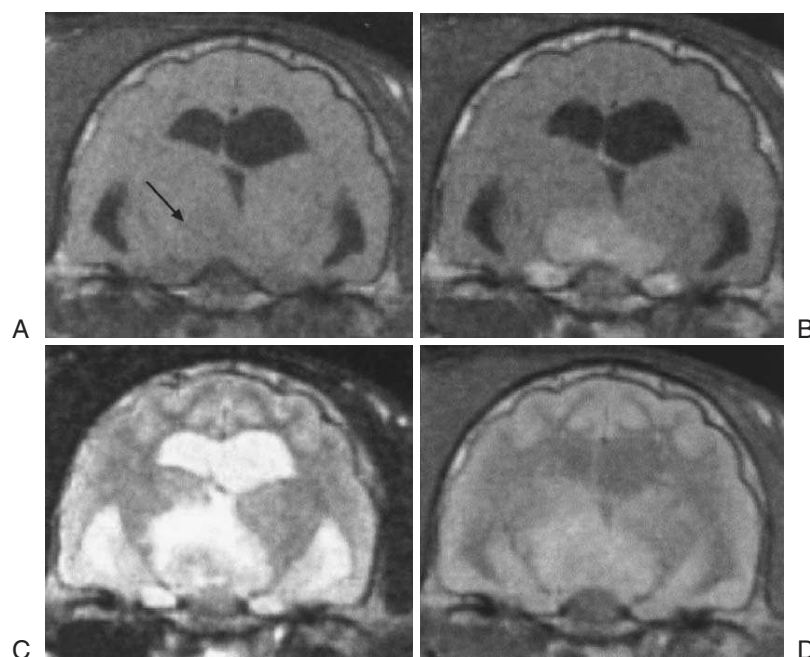
Figure 29-4

Transverse T1-weighted precontrast **(A),** T1-weighted postcontrast **(B),** T2-weighted **(C),** and proton-weighted **(D)** MR images of the brain of a 9-year-old spayed female domestic shorthair cat at the level of the midbrain. Note the slightly hypointense area at the base of the brain *(arrow)* on the T1-weighted image that is distorting the third ventricle. This area is uniformly contrast enhancing **(B).** Increased signal intensity is evident on T2-weighted images **(C)** and proton-weighted images **(D).** Immunohistochemical analysis of the mass confirmed B-cell lymphoma.

Figure 29-5

Transverse postcontrast CT image of the head of a 12-year-old spayed female miniature schnauzer dog with a history of seizures. The image is at the level of the tympanic cavities. There is a well-circumscribed, uniformly enhancing space-occupying lesion in the right temporal lobe of the cerebrum *(arrows)*. The lesion has a broad meningeal attachment and all the other CT characteristics of a meningioma. The tumor was removed surgically, and the histologic diagnosis was chemodectoma.

demonstrate dramatic improvement in clinical signs for weeks or months with sustained glucocorticoid therapy. Should seizure therapy be needed, phenobarbital or bromide are the drugs best suited for the control of generalized seizures.[73]

Four methods of therapy for a brain tumor are available at this time for use in dogs and cats: surgery, irradiation, chemotherapy, and immunotherapy.[91,117,118] The development of gene therapy strategies for treatment of intracranial tumors offers promise; however, research in this area is still at an early stage of development.[107,119] Combinations of surgery and photodynamic therapy are also being developed.[120]

Surgery

In association with the availability of CT and MRI and the development of advanced neurosurgical, anesthetic, and critical care techniques, complete or partial surgical removal of intracranial neoplasms is being practiced with increasing frequency.[121-123] Neurosurgical intervention is now an essential consideration in the management of intracranial neoplasms of cats or dogs, whether for complete excision, partial removal, or biopsy (Figure 29-7).[64]
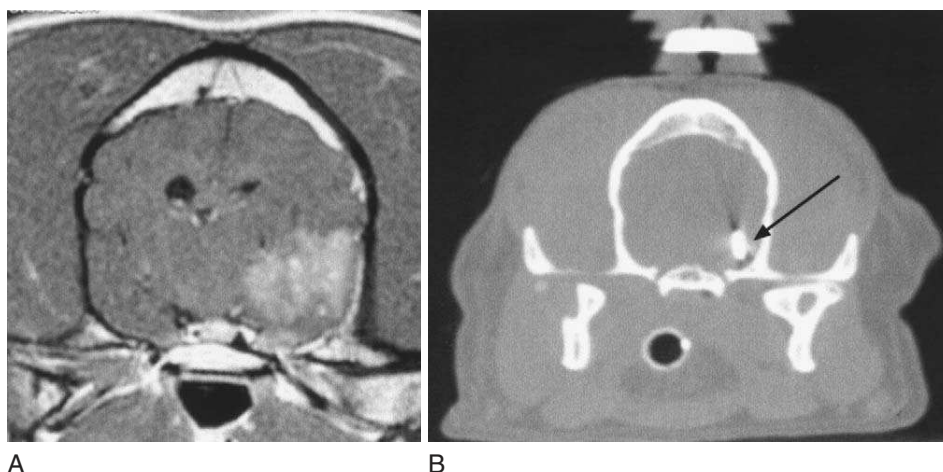
Figure 29-6

A, Transverse, postcontrast T1-weighted MR image of the brain of an 11-year-old spayed female mixed-breed dog. Note the nonuniformly contrast-enhancing mass in the left pyriform lobe of the cerebrum. A CT-guided biopsy confirmed the presence of a non-neoplastic lesion (nonsuppurative encephalitis). **B**, Transverse CT image of the brain at a similar level to the MR image shown in **(A)** confirms the location of the tip of the biopsy needle *(arrow)*.

Figure 29-7

Transverse postcontrast CT image of the head of a 9-year-old castrated male black Labrador retriever. The image is at the level of the tympanic cavities. The dog had a history of generalized seizures, blindness in the right eye, and mild right-sided postural reaction deficits. Note the irregularly enhancing space-occupying lesion in the left occipital lobe of the cerebrum. The mass was partially excised by means of a lateral craniotomy. Surgery was followed by irradiation of the brain (45 Gy in 15 fractions over 3 weeks). The dog survived 3 years after completion of irradiation. The histologic diagnosis was chondrosarcoma.

The precise location, size, and extent of a neoplasm, combined with the invasiveness of the tumor, determine the possibility of complete surgical removal. The possibility of complete excision is also affected by tumor type.[64] Meningiomas, particularly those located over the cerebral convexities or in the frontal lobes of the cerebrum, often may be completely (or almost completely) removed by means of surgery, especially in cats. In contrast, there is a significant morbidity and mortality associated with the surgical removal of neoplasms located in the caudal fossa and brain stem of cats and dogs.[22,64,117,123-125]

In addition to providing a tissue diagnosis of tumor type, partial removal of a brain neoplasm may relieve signs of cerebral dysfunction and, in turn, may render an animal a better candidate for other forms of therapy. Cytoreduction also lessens the volume of tumor available for therapy using other treatment regimes, such as radiation therapy.[46] However, surgical biopsy or cytoreduction of a tumor must be approached with care, as seeding of a tumor to previously uninvolved tissue may result in some cases.

Few data are available concerning the surgical management of secondary brain tumors. Calvarial tumors such as osteosarcomas, chondrosarcomas, and multilobular osteochondrosarcomas have been removed successfully,[21] and in the author's experience, it appears that the prognosis for tumor-free survival after the removal of a calvarial osteosarcoma may be better than that for osteosarcomas of long bones.

Intracalvarial primary bone tumors may also be removed surgically prior to other types of therapy. Surgical removal of focal metastases may also be considered in certain cases.

Radiation therapy

The use of radiation therapy for the treatment of primary brain tumors of dogs and cats is well established.[22,46,91,126-140] Irradiation may be used either alone or in combination with other treatments.[141] Radiation therapy also is recommended for the treatment of secondary brain tumors. Metastases, pituitary macroadenomas or macrocarcinomas, and skull tumors have been managed successfully by means of either radiation therapy alone or as an adjunct to surgery.[129,130,135,137] Lymphoma may also be sensitive to radiation therapy.[130]

The objective of radiation therapy is to destroy a neoplasm while minimizing damage to any normal tissue included in the irradiated volume. Improvements in treatment planning related to the use of CT and MRI have resulted in improved local control of tumors and a decrease in radiation-related problems to the CNS.[46] External beam, megavoltage irradiation currently is recommended for the therapy of brain tumors in dogs or cats.[22,46] Orthovoltage radiation has been used to treat canine brain tumors (total dose of 45 Gy in 3.75 Gy fractions over 28 days) without adverse effects.[132] Note, however, that orthovoltage radiation is not optimal because of poor beam penetration profile and limited field configuration.[132,133]

Careful treatment planning by a qualified and experienced radiation therapist is essential to the success of radiation therapy. The selection of a radiation dose is based partly on considerations such as tumor type and location and partly on tolerance of the tissues that surround the tumor (tumor bed) and have not been invaded by the tumor.[133] The response of tissues in the tumor bed may be altered by proximity to the tumor through indirect effects such as pressure, altered vascular supply, or impaired CSF drainage. When possible, multiple fractions of 3 Gy or less should be used over 3 to 5 weeks.[46] The tolerance dose to normal tissues with this fractionation schedule has been reported to be 50 to 55 Gy.[46] In the author's experience, total doses of radiation in excess of 46.5 Gy in 15 fractions over 19 days may result in late, delayed reactions, including brain necrosis. A total dose of 45 Gy given in 15 equal fractions over 3 weeks appears to be well tolerated by the tumor bed of cats and dogs. Further studies are needed to determine the optimal dose and the number of fractions that should be given as well as the time over which radiation therapy should extend. Advanced radiation delivery techniques such as intensity modulated radiation therapy and conformal avoidance such as tomotherapy (see Chapter 12) are becoming increasingly available in veterinary medicine and will ultimately enhance the effectiveness of this modality in the future.

Numerous techniques have been developed to improve the effectiveness of irradiation; however, these techniques have received only superficial attention in veterinary medicine. Superfractionation (using two or more fractions each day) has several advantages over the protocol outlined earlier for the treatment of brain tumors. However, the logistics of twice-daily fractions are difficult to organize, and since each treatment requires anesthesia of the animal, these protocols would stress a dog or cat considerably. Radiation enhancers (sensitizers) such as misonidazole also may be considered. These drugs substitute for oxygen in the hypoxic areas of a brain tumor and may render them more radiation sensitive. Boron neutron capture therapy (BNCT) is another radiation-associated therapy that is currently under investigation;[142] however, BNCT seems to be equivalent to conventional radiation therapy for treatment of intracranial tumors in dogs.[46] Hyperthermia (local or whole-body) and photoradiation therapy[143,144] may also be used to potentiate the effects of radiation therapy.

Brachytherapy, or interstitial radiation therapy, is a method used to implant radioisotope seeds (e.g., I^{125}) into the center of a tumor to deliver high doses of radiation. This technique has been used in dogs with limited success.[10,22,46]

Radiosurgery is a term used to describe the application of radiation in a highly localized nature in a single fraction.[46,139] Radiosurgery is performed through the use of numerous intersecting gamma ray beams or through the use of a large number of ports or arch therapy with a linear accelerator. Use of this technique in dogs has recently been reported.[145]

The use of a modified CT scanner to diagnose and, immediately after, to deliver accurately multiarc rotational irradiation to 25 dogs with (CT-diagnosed) intracranial masses has been described.[113] Initial results indicate marked clinical improvement in irradiated dogs, and a significant reduction in mass size, without adverse effects from irradiation.[146]

Chemotherapy

Traditionally, cytotoxic drugs have had a limited role in the treatment of dogs or cats with brain tumors, and progress in the development of truly effective chemotherapeutic protocols for humans or companion animals has been slow. Several factors affect the use of chemotherapeutic agents for the treatment of brain tumors in dogs or cats.[147] The first, unique to the brain, is that the blood-brain barrier may prevent exposure of all or some of the tumor to a chemotherapeutic agent injected parenterally. Second, tumor cell heterogeneity

may be such that only certain cells within a tumor are sensitive to a given agent. Third, a tumor may be sensitive only at dosages that are toxic to the normal brain or other organs. Methods for increasing drug delivery to the CNS are under investigation for use in humans. Intra-arterial administration of drugs, high-dose systemic therapy, and blood-brain barrier disruption are all currently under investigation in animal models.[148,149]

Many types of CNS malignancies differ in their response to cytotoxic drugs.[150] Certain CNS tumors in humans, such as CNS lymphoma, medulloblastoma, and oligodendroglioma, may be remarkably chemosensitive.[150] Future directions that might enhance the use of chemotherapeutic agents in dogs or cats with brain tumors include transient osmotic blood-brain barrier disruption,[151,152] or transient opening of the blood-brain barrier using bradykinin analogs such as RMP-7.[153]

Cytosine arabinoside (ARA-C) has been used intrathecally in dogs to treat CNS lymphoma.[60] Investigation has shown that ARA-C penetrates the intact canine blood-brain barrier and that cytotoxic concentrations may be achieved in the CNS following intravenous administration.[154] Both carmustine (BCNU) and lomustine (CCNU) have reduced tumor size, and improved clinical signs, in dogs with glial cell tumors.[117,147,155,156]

Immunotherapy

An approach that mobilizes cell-mediated immunity against a brain tumor by culturing and stimulating autologous lymphocytes and then returning them to the tumor bed following tumor resection has been utilized in dogs.[157] Reduction in tumor size and clinical improvement occurred in five dogs with cerebral gliomas. The treatment of dogs with meningiomas using repeated intracisternal injections of stimulated lymphocytes also resulted in clinical improvement and reduced tumor size.

Gene therapy

Gene therapy may be defined as a treatment modality in which DNA or RNA is transferred to target cells to modify their genetic makeup for therapeutic purposes.[107,158] Although gene therapy was originally envisioned as a means to treat genetic diseases, its potential use in the treatment of cancer was quickly recognized. Examples of this include enhancing the immunogenicity of a tumor by introducing genes that encode foreign antigens or genes that encode cytokines, inserting a "suicide" gene into a tumor, blocking the expression of oncogenes, and inserting a wild-type p53 gene into p53-deficient tumor cells.[152] Other suitable targets for therapy are the regulators of angiogenesis.[159] Angiogenesis modulators are extremely important in

tumor growth. Angiogenesis inhibitors may eventually be used to augment conventional therapy and help to prolong dormancy of microscopic metastases.

To date, most of the approved human clinical gene therapy protocols involve cancer therapies, and several of these are designed to treat brain tumors.[160] The failure of conventional therapy to cure malignant brain tumors, the toxicity of these therapies, and the identification of many of the genetic abnormalities associated with brain tumors are helping to drive interest in gene therapy to treat brain tumors. Although the treatment success achieved in mouse and rat brain tumor models has not translated to current clinical studies in human subjects, the results are encouraging and warrant further development of gene therapy for brain tumors.[107] Poor transfection of the tumor mass appears to be the primary obstacle to large-scale destruction of targeted tumors. Another hurdle to overcome is maximizing delivery of genetic material to the tumor. Routes of administration currently being investigated include stereotactic intratumoral injection and intra-arterial vector administration with or without disruption of the blood-brain barrier.[161] Future studies will involve better systems for more efficient and specific delivery of the genetic material to the tumor and methods to enhance the patient's immune system response.[160]

Prognosis

Few data exist concerning the survival times of dogs or cats with a brain tumor that have received only palliative therapy (i.e., therapy to control the secondary effects of a tumor without an attempt to eradicate the tumor). The results of one study indicate a mean and median survival of 81 days and 56 days, respectively, following CT diagnosis of a primary brain tumor in each of 8 dogs.[126] Six of the 8 dogs in this study died or were euthanatized within 64 days of brain tumor diagnosis, though 1 dog survived for 307 days, indicating that survival times are difficult to predict.[126] In another study that included 13 dogs with intracranial meningiomas, survival times from initial clinical signs to necropsy varied from 1 day to 405 days, with a mean survival time of 75 days.[71] In the same study, the survival times for 7 dogs with astrocytoma were from 7 days to 150 days, with a mean survival time of 77 days. In a report of 86 dogs with either primary or secondary brain tumors, the median survival time for 45 dogs receiving either no treatment (7 dogs) or symptomatic treatment (38 dogs) was 6 days.[22]

Two more recent papers on the results of primary irradiation of brain tumors in dogs are encouraging with median survivals of 250 to 699 days.[138,140] Both papers suggested that there were no predictive indicators of

outcome, acute and late effects were mild, and in most cases the cause of death was local recurrence.

The results from several studies confirm that the prognosis for a dog or cat with a primary brain tumor may be significantly improved by surgical removal, irradiation, chemotherapy, or immunotherapy, used either alone or in combination.[22,64,117,132] In a retrospective study in which 41 dogs, with either primary or secondary brain tumors, received some form of definitive treatment (i.e., surgery, Cobalt-60 irradiation, whole-body hyperthermia, ^{125}I implants, and chemotherapy, alone or in combination), the factor most associated with survival time was mode of therapy.[22] Dogs that were treated with irradiation, with or without other combinations of therapy, lived significantly longer than those that received surgery (with or without ^{125}I implants) or those that received symptomatic therapy. In this same study, the main indicators for poor prognosis, after adjusting for treatment, were involvement of multiple brain regions and moderate-to-severe neurologic signs. Other factors that negatively influenced survival in this study included increased white blood cell count in CSF, a rapid and progressive clinical course, tumor types other than meningioma, and secondary brain tumors.

The reports concerning surgical removal alone to treat the primary brain tumors of dogs have dealt primarily with superficial, rostrotentorial meningiomas. In one study of 4 dogs that had surgical removal of olfactory meningiomas, postoperative survival times were 63 days to 203 days, with a mean survival time of 138 days.[162] In another study, 10 dogs treated surgically for intracranial meningiomas had a median survival time of 198 days, with a 1-year survival rate of 30%.[64] It appears that dogs have an excellent prognosis for long-term survival following complete surgical excision of a solitary cerebral meningioma. "Complete" removal should be confirmed following careful histologic examination of tumor margins by a pathologist. The prognosis for dogs or cats after surgery alone for removal of a meningioma is affected by the occurrence of postoperative complications, such as infection, cerebral edema, or hemorrhage.

Irradiation, alone or in combination with surgery, has been used frequently to treat canine brain tumors. In a review of four reports[22,126,131,163] of radiation therapy, alone or with surgery, used as a treatment for canine brain masses (i.e., both primary and secondary brain tumors), the mean and median survival times ranged from 160 days to 433 days, and 160 days to 360 days, respectively.[132] A median survival time of 345 days was reported for 14 dogs with a variety of brain masses that received orthovoltage irradiation.[132] In a study of megavoltage irradiation of pituitary macrotumors of dogs, the only factor that significantly affected survival time was the severity of neurologic signs.[135] Mean and median survival times for dogs with severe neurologic signs in

this study were 17 ± 3 months and 13.3 ± 8.3 months, respectively, and the survival times for dogs with mild neurologic signs were 20 ± 3.5 and 21 ± 2.8 months, respectively. In most animals, radiation therapy should commence within several days of surgery if the animal is clinically stable and able to survive anesthesia. Use of the optimum energy (linear accelerator) coupled with detailed treatment planning and fractions less than or equal to 3 Gy provide the best improvement in results compared with past experience.[46]

Dogs that have been treated for a brain tumor may develop a second type of tumor elsewhere in the body. For example, in six dogs with pituitary macrocarcinomas or macroadenomas treated by means of irradiation, three developed second tumors outside the original treatment field within 2 years of the completion of therapy.[129]

A number of reports concern the surgical removal of feline meningiomas.[64,74,124,125,164] All confirm that surgical removal of a solitary meningioma in cats results in excellent long-term survival. In a series of 10 cats treated by surgical removal of a meningioma, 1 cat died immediately after surgery, and 3 cats died of concurrent disease unrelated to cerebral neoplasia 2 to 30 months after surgery.[74] In another study, 4 cats that had surgical removal of a solitary meningioma had a mean survival time of 485 days, with 2 cats surviving more than 2 years after surgery (50% survival at 2 years).[64] A report of 17 cats with meningioma treated by surgical removal indicates that 3 died in the immediate postoperative period, 3 cats died with tumor recurrence at 3 months, 9 months, or 72 months after surgery, and the remaining 11 cats survived without tumor recurrence for 18 months to 47 months (median 27 months) postoperatively.[124] The results of craniotomy for the treatment of cerebral meningioma in 42 cats indicate an overall survival of 71% at 6 months, 66% at 1 year, and 50% at 2 years.[125] In another study, radiation therapy resulted in excellent long-term survival in 2 of 3 cats with pituitary tumors and acromegaly.[136]

Comparative Aspects

Brain tumors amount to less than 2% of all malignant neoplasms in humans, although a significant proportion of CNS neoplasms affect children, in which tumors of the nervous system rank second in incidence after leukemias.[34] New brain tumors develop in approximately 35,000 adult Americans each year.[165] In children, astrocytomas and medulloblastomas are the most common tumors; in adults, the most common are metastatic tumors, astroglial neoplasms (including glioblastoma multiforme), meningiomas, and pituitary adenomas. Evidence suggests that the incidence of primary tumors among the elderly is increasing. Malignant gliomas account for 2.5% of the deaths due to cancer and are the third leading cause of death from cancer in persons 15 to 34 years of age.

Little is known about the relationship of environmental factors to primary brain tumors. Cranial irradiation and exposure to certain chemicals may increase the incidence of both astrocytomas and meningiomas. Head injury may potentiate meningiomas but does not appear to cause astrocytomas. Sixteen percent of patients with primary brain tumors have a family history of some type of cancer.[165,166]

The morbidity and mortality associated with brain tumors in humans vary with the histologic type. Only 10% of patients with malignant astrocytoma or glioblastoma multiforme are alive 18 to 24 months after therapy, regardless of the type of therapy used. Some combination of surgery and irradiation is required to achieve 50% to 70% 5-year survival rates in virtually all the adult cases of low-grade astrocytoma, oligodendroglioma, or ependymoma. Approximately 80% of patients are free of recurrence 5 years after a meningioma has been excised, and 50% are free of tumor 20 years after surgery. The only factor significantly associated with recurrence-free survival following resection of a meningioma is the completeness of surgical excision. Irradiation successfully improves the survival of patients that have had incomplete surgical resection of a meningioma.[165,166]

SPINAL CORD NEOPLASIA

Incidence and Risk Factors

Tumors affecting the spinal cord may be classified as extradural, intradural-extramedullary, and intramedullary.[1] Extradural neoplasms comprise approximately 50% of all spinal neoplasms, while intradural-extramedullary tumors and intramedullary tumors constitute 30% and 15%, respectively.[1,167] The reported series are not sufficiently large to yield reliable data concerning age, breed, or sex predilections.[168] In one study, 8 of 29 dogs or cats (27.6%) were 3 years of age or younger, and 90% of the tumors occurred in large-breed dogs.[169]

Primary spinal cord neoplasms occur infrequently in dogs. Meningiomas appear to be the most frequently diagnosed primary spinal neoplasm of dogs.[1] Such tumors have a high incidence in the cervical spinal cord.[1] A primary spinal cord neoplasm with a high incidence in young dogs (6 months to 3 years of age) is the intradural-extramedullary spinal cord tumor (see Box 29-1).[170-176] Hemangiosarcoma is the most common secondary tumor affecting the canine spinal cord.[177]

Primary spinal cord neoplasia, with the exception of lymphoma, is relatively rare in cats.[30] Lymphoma was diagnosed in 214 of 1897 necropsies done on cats in one study.[30,178] Of these 214 cats, there was CNS involvement in 26 (21.1%). Twenty-three of these 26 tumors involved the spinal cord, and 22 of the 23 were solitary. A predilection for the thoracic and lumbar vertebral canal was noted. Most cats with spinal lymphoma were young (median age 24 months). In another series of 150 cats with lymphoreticular malignancies, 8 (5.3%) had gross CNS involvement.[179] Signs of neurologic dysfunction were recognized in 10 (10.9%) of 92 cats with lymphoma in another report.[30] In a series of 205 cats with spinal cord disease, lymphoma was the most common disease affecting cats between 2 and 8 years of age, while vertebral column neoplasia (osteosarcoma, fibrosarcoma, undifferentiated sarcoma and plasmacytoma) were the most common diseases in cats greater than 8 years.[180]

Etiologic factors of spinal tumors are poorly defined in cats and dogs. In cats, feline leukemia virus (FeLV) is frequently associated with lymphoma and therefore must be considered a risk factor in the development of spinal lymphoma.[178,181,182] However, a cat with spinal lymphoma may test negative for FeLV. Spinal lymphoma has also been associated with feline immunodeficiency virus infection.[183]

Pathology

In dogs, the most frequently reported extradural tumors are primary malignant bone tumors (osteosarcoma, chondrosarcoma, fibrosarcoma, hemangiosarcoma, hemangioendothelioma, and myeloma) and tumors metastatic to bone and soft tissue.[2,169,184,185] Secondary tumors affecting the canine vertebrae arise from numerous primary tumor types.[2,169,184,186] Metastatic extradural neoplasms are unusual in dogs; however, extradural lymphomas and liposarcomas have been reported.[1,187,188]

Meningiomas and peripheral nerve sheath tumors are the most frequently occurring intradural-extramedullary neoplasms of dogs.[168] These tumors are reported to occur most frequently in older dogs of either gender. In one report, approximately 14% of CNS meningiomas involved the spinal cord in dogs (40% cervical, 32% thoracic, and 28% lumbar spinal cord).[169] In another study of spinal meningiomas in 13 dogs, 3 were in the lumbar region, and 10 were located in the cervical region.[168] A review of nerve sheath tumors revealed that 39 of 60 tumors constituted the spinal cord.[14] Peripheral nerve sheath tumor was the most common tumor in another review of 30 tumors affecting the spinal cord.[184]

A less frequently reported spinal cord neoplasm is the intradural-extramedullary spinal cord tumor of young dogs, also classified as neuroepithelioma, ependymoma, medulloepithelioma, nephroblastoma, and spinal cord blastoma.[176] This neoplasm has a predilection for the T10 through L2 spinal cord segments of young dogs, especially German shepherd dogs and retrievers.[170-176]

Intramedullary spinal tumors of dogs occur infrequently.[2] They are predominantly of glial cell origin;

astrocytoma, oligodendroglioma, undifferentiated sarcoma, ependymoma, and choroid plexus papilloma have been reported.[2] Granulomatous meningoencephalomyelitis may also occur as a primary spinal cord lesion. Intramedullary spinal cord metastases may occur in dogs with systemic malignancy, in the absence of tumor metastasis in the epidural space or vertebral bone, and as the first clinical manifestation of cancer.[2,177,189] Hemangiosarcoma and lymphoma show a propensity for intramedullary spinal cord involvement.[177] Intramedullary metastases also have been reported in association with mammary gland adenocarcinoma and malignant melanoma.[1] Occasionally, malformation tumors may affect the spinal cord (e.g., epidermoid cyst).[2]

Extradural lymphoma, either primary or secondary, occurs frequently in cats.[178,182] Of feline extradural tumors, osteosarcoma is the most common primary vertebral neoplasm. Single or multiple osteochondromas have been identified in several cats.[30,180] Of the intradural-extramedullary tumors, meningiomas have been infrequently reported in the spinal cord of cats. Intramedullary tumors of cats are extremely rare and usually have features consistent with primitive neuroectodermal tumors or are of glial origen.[30,180]

History and Clinical Signs

Extramedullary spinal cord neoplasms typically are slow growing and gradually compress the spinal cord. The signs of spinal cord dysfunction usually worsen over weeks or months. Occasionally, an acute onset of signs may accompany hemorrhage or ischemia associated with a neoplasm. Intramedullary tumors, which may grow more rapidly, are characterized by a higher incidence of ischemia, necrosis, and hemorrhage.

The clinical signs associated with a spinal cord tumor usually reflect the location of the neoplasm and often are indistinguishable from the signs caused by other transverse myelopathies at the same location.[184] The presence of certain signs should cause suspicion of a spinal cord neoplasm. Extradural tumors may involve the meninges, spinal nerves, or nerve roots, resulting in discomfort that may progress to extreme spinal hyperesthesia.[184] Neurologic deficits (e.g., paresis) may not be evident initially and when present may be intermittent (i.e., worsen with exercise). Usually, neurologic function progressively deteriorates caudal to the lesion. Intradural-extramedullary tumors may also result in a prolonged, intermittent expression of clinical signs and hyperesthesia; however, the signs may be alleviated by exercise.[168] Brachial or lumbar intumescence involvement may be evidenced by lameness, holding up of a limb, neurogenic muscular atrophy, and depressed spinal reflexes. Rarely, unilateral spinal cord compression may cause deficits in the contralateral limb.

In contrast, intramedullary spinal cord tumors usually hasten neurologic dysfunction.[169] Hyperesthesia rarely is associated with such tumors.[190]

Diagnostic Techniques and Workup

The diagnosis of a neoplasm affecting the spinal cord requires a systematic approach.[191] The procedure is based on collecting and interpreting a minimum database that includes appropriate serologic tests (hemogram, biochemical profile) and thoracic radiographs for primary or metastatic neoplasia. Following this step, survey radiographs of the vertebral column, CSF collection and analysis, and myelography or advanced imaging may be completed during a single period of anesthesia.

Radiography

General anesthesia permits accurate positioning of a dog or cat for survey radiographs of the vertebral column and allows stressed or oblique projections to be done. Primary or secondary vertebral tumors may produce bone lysis, new bone production, or both. The vertebral body and arch are more frequently affected by a neoplasm than the dorsal spinal processes or transverse processes. Plain radiographic abnormalities are uncommon with primary nervous system neoplasms. The expansion of a spinal tumor may result in the enlargement of an intervertebral foramen, widening of the vertebral canal, or thinning of surrounding bone (Figure 29-8).

Cerebrospinal fluid analysis

Cerebrospinal fluid collection and analysis are indicated when plain radiographs do not provide a complete diagnosis. A lumbar puncture is recommended for CSF collection, and the needle may be left in place for myelography, pending the results of the cytologic examination of CSF. The alterations in CSF caused by spinal tumors should be interpreted according to the same criteria discussed for brain tumor diagnosis;[76] however, it must be remembered that the protein content of CSF collected from a lumbar location is normally higher than that of CSF collected from the cerebellomedullary cistern.[82] Lymphoma affecting the spinal cord often results in an elevated white cell count, predominantly abnormal lymphocytes.[178,182]

Myelography

Myelography can accurately determine the location and extent of a spinal cord neoplasm. On the basis of myelography, the tumors may be classified as extradural (Figure 29-9), intradural extramedullary (Figure 29-10), or intramedullary (Figure 29-11), although this distinction cannot always be made. For example, it is not always possible to distinguish between an intramedullary and an intradural-extramedullary lesion on
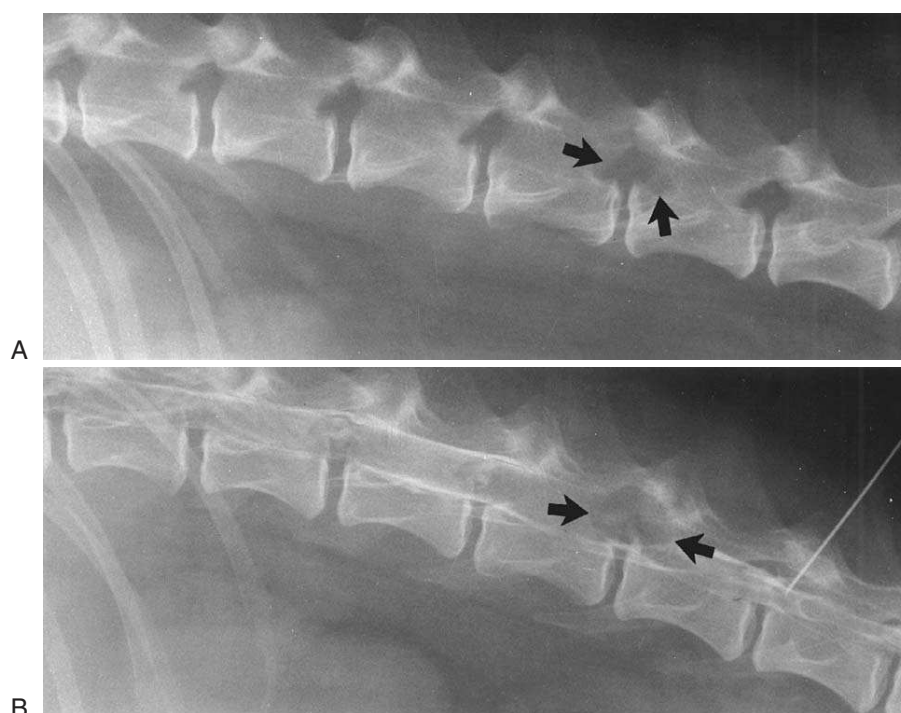
Figure 29-8

A, Lateral radiograph of the lumbar vertebral column of a 10-year-old spayed female Australian shepherd dog with a 3-week history of progressive lameness in the left pelvic limb. Note the lysis surrounding the L4 to L5 intervertebral foramen *(arrows)*. **B**, Myelogram of the lumbar region dog in **A.** There is a filling defect at the level of the L4 to L5 intervertebral foramen, consistent with a space-occupying lesion *(arrows)*.

the basis of myelographic findings. In such cases, CT or MRI may provide more exact localizing information (Figure 29-12).[97,100,192]

Advanced imaging

The use of CT and MRI for imaging of the vertebral column and spinal cord is increasing.[193,194] CT may provide information regarding cortical bone detail that is not available by means of plain radiography or myelography (Figure 29-13), while MRI provides a safe and noninvasive method for evaluating the spinal cord and related structures (Figure 29-14).[193,194]

Treatment

A limited number of therapeutic options exist for a dog or cat with a spinal cord neoplasm.[195] Appropriate therapy depends on tumor location, extent, and histologic type. An immediate goal of therapy is to relieve the deleterious effects of sustained spinal cord compression. This may be achieved medically (e.g., glucocorticoids) or surgically.[191] Surgery may permit the complete removal or cytoreduction and biopsy of a neoplasm.[168,196-198] When complete removal is not possible, recurrence is to

be expected, and adjunctive therapy such as irradiation is recommended.[187,197,199] However, the primary problem with spinal cord irradiation is that with conventional therapy, the spinal cord must be irradiated to the same dose as the tumor.[46] The development of advanced neurosurgical techniques and the introduction of new biopsy methods have improved the outcome in many cases.[167,182,200-202]

Accurate biopsy diagnosis of lymphoma is essential, because lymphoma of the spinal cord may be successfully treated with chemotherapy or irradiation alone or in combination.[60,178,182] Intraoperative smear preparations of spinal cord tumors may circumvent the need for extensive surgical debulking if a lymphoma is diagnosed. In certain cases, radiation therapy may be done prior to chemotherapy to effect a rapid reduction in tumor mass.[60]

Prognosis

Few reports in the veterinary literature concern the long-term follow-up of dogs and cats with spinal neoplasia.[167,195] The prognosis depends on the resectability, histologic type, location, and severity of clinical signs.[167]

A

B
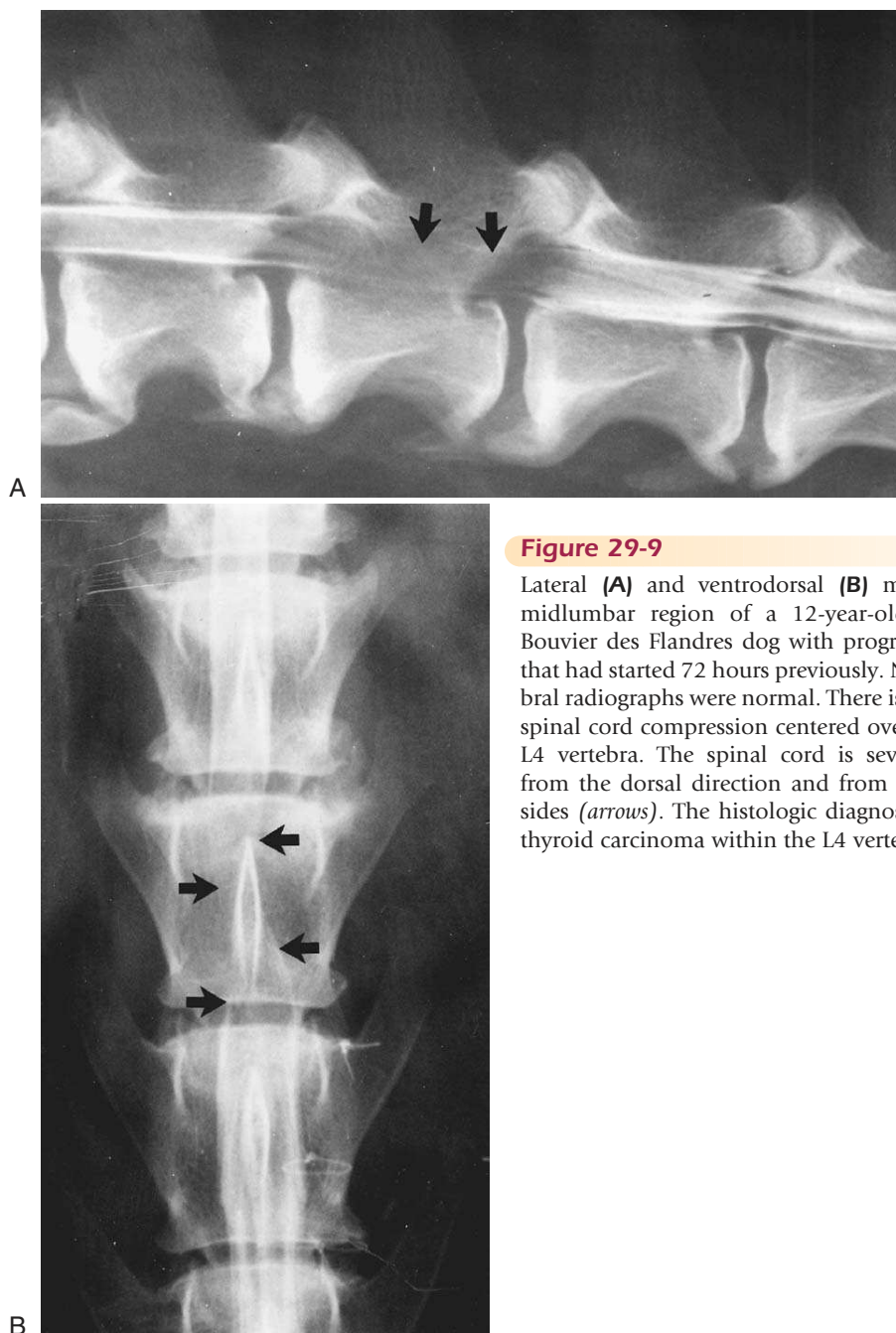
Figure 29-9

Lateral **(A)** and ventrodorsal **(B)** myelogram of the midlumbar region of a 12-year-old spayed female Bouvier des Flandres dog with progressive paraparesis that had started 72 hours previously. Noncontrast vertebral radiographs were normal. There is severe extradural spinal cord compression centered over the body of the L4 vertebra. The spinal cord is severely compressed from the dorsal direction and from the left and right sides *(arrows)*. The histologic diagnosis was metastatic thyroid carcinoma within the L4 vertebra.

Generally, dogs or cats with an extradural metastatic neoplasm or vertebral neoplasm have a poor prognosis, and palliative therapy only is attempted.[195,195a] Removal of an affected vertebra (spondylectomy), particularly in the cranial lumbar region, may be attempted in selected cases.[202] Occasionally, intradural-extramedullary tumors may be completely resected, and in such cases the prognosis must be considered good. In one study, five of nine dogs survived for longer than 6 months following the surgical resection of a meningioma.[168]

Of the five dogs, one was alive 3 years after surgery. Few data exist concerning irradiation or chemotherapy of spinal cord neoplasms in animals; however, such therapies should be considered.[178,182] The author has treated several intramedullary tumors of dogs (including ependymoma and lymphoma) by means of irradiation. The results in these cases indicate that the canine spinal cord tolerates irradiation well and that the clinical signs of spinal cord tumors may be alleviated for more than 1 year.
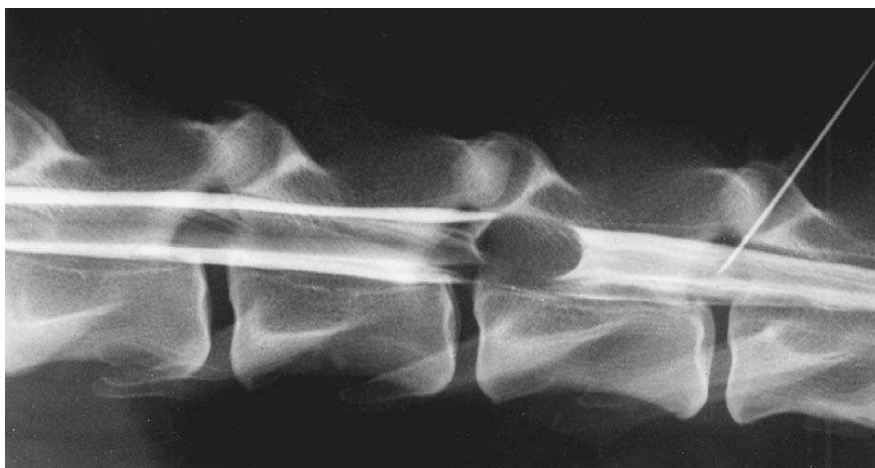
Figure 29-10

Lateral myelogram of the lumbar region of the vertebral column of a 10-year-old spayed Labrador retriever. The dog had progressive paraparesis of several months' duration. Noncontrast vertebral radiographs were normal. Note the well-defined intradural-extramedullary lesion at the level of the L5 to L6 vertebrae. The mass was removed surgically and the histologic diagnosis was meningioma. The dog made a complete recovery and was normal 6 months following surgery.
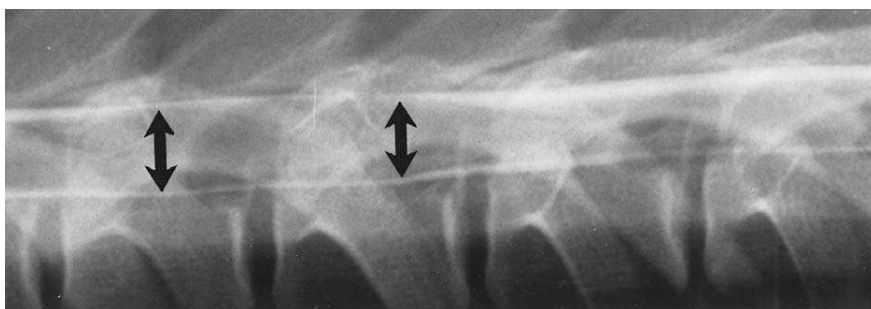
Figure 29-11

Lateral myelogram of the thoracic vertebral column of a 3-year-old spayed Labrador retriever with progressive paraparesis of 6 months' duration. Plain radiographs of this region were normal. The lateral myelogram outlined an expansile intramedullary lesion at the level of the T5 to T6 vertebrae *(arrows)*. The dog's signs continued to worsen, and 2 months following this study, the dog was euthanatized. An astrocytoma of the spinal cord was confirmed at the level of the T5 to T6 vertebrae.

Comparative Aspects

Intradural spinal cord neoplasms are uncommon in humans; their incidence is from 3 to 10 in a population of 100,000.[203] The ratio of intradural to extradural tumors is approximately 3:2. These tumors occur predominantly in the middle decades of life, and except for an unusually high incidence of meningiomas in females, the sex incidence is approximately equal. Of the intradural-extramedullary tumors, peripheral nerve sheath tumors constitute about 30%, and meningiomas constitute about 25%.

Astrocytoma and ependymoma, each with a similar incidence, are the most common intramedullary tumors. Approximately 90% of all intradural spinal cord tumors in humans are benign and potentially resectable. Therefore, in contrast to animals, humans may have an excellent prognosis following surgical therapy.

In humans the majority of extradural spinal cord tumors are metastases. Approximately 5% of cancer patients develop spinal epidural tumors, although not all of these become clinically evident. Primary CNS tumors such as neurofibromas or meningiomas occasionally may be limited to the extradural space.

A

B
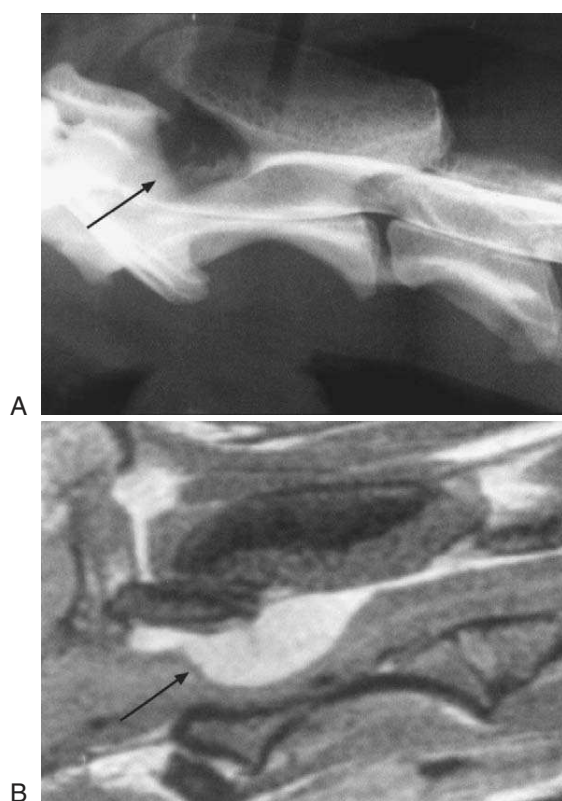
Figure 29-12

Lateral myelogram **(A)** and parasagittal postcontrast T1-weighted MR image **(B)** of the cranial cervical vertebral column of a 6-year-old spayed female cocker spaniel. The myelogram **(A)** demonstrates an intradural-extramedullary mass *(arrow)* dorsal to the spinal cord at C1 to C2. The exact extent of this mass *(arrow)* can be determined from the MR image **(B)**. The mass was a meningioma. The dog is alive 12 months following surgical removal of the mass and radiation therapy.

Figure 29-13

Transverse precontrast CT image of the vertebral column of a 7-year-old spayed Australian shepherd dog at the level of the L4 vertebra. There is a large tumor arising from the right transverse process of the L4 vertebra. The tumor involves the body of this vertebra. Note the soft tissue mass crossing the midline ventrally *(arrowheads)*. The histologic diagnosis was osteosarcoma.

The prognosis for humans with extradural spinal neoplasms depends on the histologic type, rate of onset, and severity of symptoms. A correlation between pretreatment motor status and functional outcome emphasizes the value of early diagnosis and treatment of such neoplasms. In humans, palliation is the goal when managing patients with spinal metastasis. Radiation therapy and surgical decompression individually or in combination may be used for this purpose.[203]

TUMORS OF PERIPHERAL NERVES

Incidence and Risk Factors

Primary neoplasms of the peripheral nerves occur relatively infrequently in domestic animals, although they have been reported in dogs, cats, cattle, goats, sheep, and pigs.[204,205] The primary tumors of the cranial and spinal nerves and nerve roots are common in dogs.[206] Metastasis of nerve sheath tumors is rare, although pulmonary metastasis has been reported.[206] Neurofibromatosis has been reported in dogs.[207]

Of 60 peripheral nerve sheath tumors of dogs of 23 breeds reported in one study, 4 involved cranial, 39 involved spinal, and 17 involved the peripheral nerves.[14] The age distribution was from 2 to 17 years, with 43 tumors occurring between 5 and 12 years of age. Forty-two dogs were male, and 18 were female. In the same study, 4 dogs with intraparenchymal schwannomas (1 of the brain, 3 of the spinal cord) were reported. In a review of 42 brachial plexus tumors of dogs described in the literature, 20 dogs (48%) had evidence of spinal cord invasion, and in 4 of these dogs, the tumor appeared to arise primarily in the dorsal root, within the vertebral canal.[208] The remaining 16 dogs in this review appeared to have brachial plexus tumors that originated peripheral to the vertebral canal and that only invaded the spinal cord as a terminal event.

In another review of 51 dogs with nerve sheath tumors, the age at diagnosis ranged from 3 to 13 years.[206] Thirty of the affected dogs in this study were male, and 21 were female. Eight dogs had tumors involving nerves distal to the brachial or lumbosacral plexus, 20 dogs had involvement of the brachial of lumbosacral plexus, and 23 dogs had tumors that involved the vertebral canal.

Primary nerve sheath tumors have been reported infrequently in cats, although solitary nerve sheath
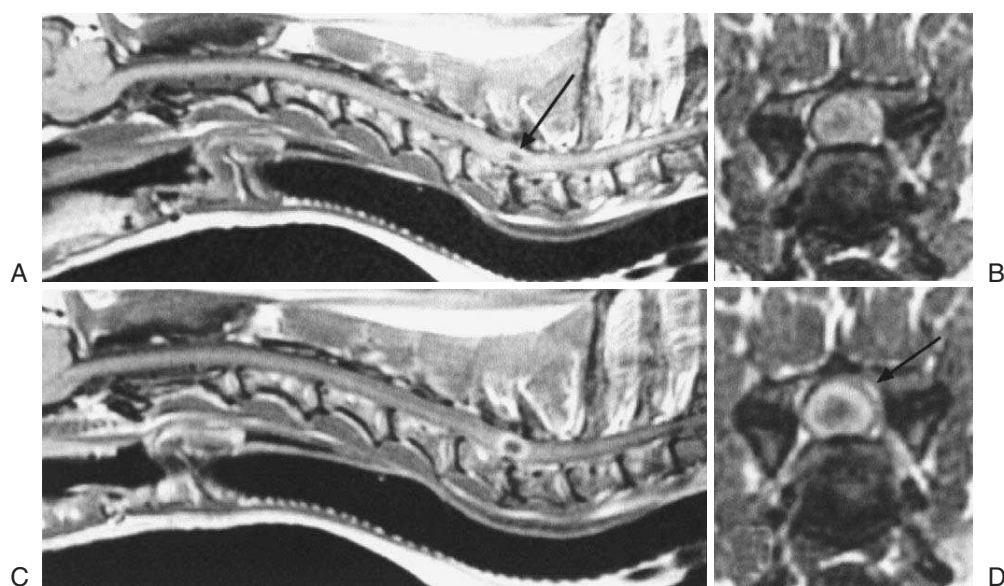
Figure 29-14

Parasagittal precontrast **(A)** and postcontrast **(C)** T1-weighted MR images of the cervical spine of a 13-year-old spayed female mixed-breed dog. An intramedullary hyperintense mass *(arrow in a)* with a hypointense central region is evident on the precontrast parasagittal image **(A)**. The periphery of this mass demonstrates uniform contrast enhancement **(C)**. Transverse precontrast **(B)** and postcontrast **(D)** T1-weighted MR images accurately demonstrate the intramedullary location of the mass, with severe compression of adjacent spinal cord *(arrow in d)*. The mass was a spindle cell tumor. *(Photographs courtesy of Dr. Craig Bergstrom.)*

tumors may be seen in this species.[9,208] A solitary nerve sheath tumor has been described in the skin of a cat and in a thoracic vertebra in another cat.[25,29]

Neuronal and nerve cell tumors arise most frequently from the sympathetic and paraganglionic components of the autonomic nervous system. Those arising from the sympathoblasts of the sympathetic system are neuroblastomas, while the paraganglionic system gives rise to paragangliomas, the best known being pheochromocytoma and chemodectoma.[209] Such tumors occur infrequently in dogs and cats.[29,201,210]

Of secondary tumors that involve the cranial and spinal nerves and nerve roots of dogs or cats, lymphoma is the most common.[25,30,182,211-214] Lymphoma has been reported in the cranial and peripheral nerves of cats.

Pathology

Primary peripheral nerve tumors may affect cranial nerves, peripheral nerves, sympathetic nerves and ganglia, and adrenal gland nerves. Most tumors of the peripheral nerves, excluding those of the sympathetic nervous system, result from neoplastic transformation (usually benign) of periaxonal Schwann cells.[206,215] Such tumors are called schwannomas.[216] They have also been called neurilemmomas or neurinomas, because they arise in a nerve or nerve sheath. The terms *neurofibroma* and *neurofibrosarcoma* have been used to describe tumors thought to be derived from endo- or epineurally located fibroblasts within the nerve sheath. Peripheral nerve sheath tumors have also been called perineural fibroblastomas, based on a theory that they arise from perineural fibroblasts.[216] Other less frequently used terms include *Schwann cell tumor*, *lemmoma*, and *lemnocytoma*.[217]

The classification of nerve sheath tumors has been widely disputed.[41,215,218,219] A controversy concerning nomenclature hinges on whether such tumors arise from Schwann cells, which produce myelin, or the fibroblasts that are responsible for producing the endo-, peri-, and epineurium. The most reliable classification is based on a combination of ultrastructural features and immunocytochemical demonstration of cell-specific marker proteins.[217] On this basis, evidence has not been found for an endo-, peri-, or epineural derivation of nerve sheath tumors; therefore, the term *schwannoma* or *nerve sheath tumor* is recommended.[206,220,221]

For each benign form of peripheral nerve sheath tumor, there is a corresponding rare but distinct malignant form in which the cells of origin become anaplastic and on occasion may invade neighboring structures or metastasize.[221] Invasion and metastases are most common with tumors of the sympathetic nervous system.

Malignant nerve sheath tumors, which are infrequent, are characterized by mitosis, cellularity, anaplasia, and, rarely, metastasis to the lymph nodes or lung.[220]

Nerve sheath tumors of dogs most frequently occur in brachial plexus nerves (C6–T2), although other nerves may be affected.[208] Secondary extension by means of nerve roots into the spinal canal may compress the spinal cord. These tumors also commonly affect cranial nerves;[2,219] the vestibulocochlear nerve and trigeminal nerve are frequently affected.[219,222] Occasionally the oculomotor nerve, or other cranial nerves, may be affected. Nerve sheath tumors occur as nodular or varicose thickenings of large nerves and usually are firm, white to gray in color, and well circumscribed (Figure 29-15).

Granular cell myoblastoma has been described in the peripheral nerves as a growth of Schwann cells.[41,223] It is not clear whether this tumor is a true neoplasm or a lipid disturbance of Schwann cells. These tumors have been reported in the tongue of dogs and may also occur in the CNS as primary tumors.[41]

History and Clinical Signs

The signs of peripheral nerve sheath neoplasia reflect the location of the neoplasm, which may involve a solitary cranial or peripheral nerve, spinal nerve or nerve root, several cranial or peripheral nerves, a plexus, or multiple spinal nerves or nerve roots. Peripheral nerve sheath neoplasms are slow growing, and the signs usually progress over weeks to months. Occasionally, signs may develop rapidly.[224]

The brachial plexus or C6 through T2 spinal nerves and nerve roots are the most common sites for peripheral nerve

Figure 29-15

Surgical exposure of the brachial plexus of a 5-year-old male Airedale terrier with a 3-month history of progressive right thoracic limb lameness and right axillary pain. Note the thickening of the nerve *(arrows)*. The histologic diagnosis was schwannoma.

sheath tumors.[225] This tumor always should be suspected in adult dogs with unilateral thoracic limb lameness of obscure cause.[206,208] With progression of the neoplasm, paresis of the affected limb and atrophy of muscles innervated by the involved nerves may develop. An animal may hold an affected limb off the ground. The passive movement of the limb or neck may cause apparent pain to the animal, and a pain response can be elicited by deep palpation cranial to the first rib. Occasionally, a palpable enlargement of a nerve or plexus is apparent. Ipsilateral Homer's syndrome, either partial (miosis only) or complete (miosis, ptosis, enophthalmos, third-eyelid prolapse), may occur, whereby the T1 or T2 nerve roots are involved.[226] Ipsilateral loss of the panniculus reflex also may be detected. The brachial plexus nerve sheath neoplasms frequently extend proximally through an intervertebral foramen to compress the spinal cord.[204,227] The signs of spinal cord involvement usually follow a chronic period of thoracic limb lameness and progressive muscle atrophy, although they may precede, or occur concurrently with, thoracic limb lameness and atrophy. Myelopathy usually results in asymmetric pelvic limb paresis. The contralateral thoracic limb may or may not be affected.

Nerve sheath tumors of the lumbosacral plexus most often cause progressive lameness of a single pelvic limb.[204] The progression of signs is similar to that described for nerve sheath tumor of the brachial plexus; however, apparent pain is less consistently seen in association with lumbosacral plexus nerve sheath neoplasms. Urinary or fecal incontinence may be present in those dogs when the tumor invades the spinal canal. Contralateral limb involvement may also be seen late in the course of the disease.

Involvement by a nerve sheath tumor of a solitary nerve root, spinal nerve, or cranial nerve results in neurologic deficits that reflect the structure involved. Should a nerve sheath tumor of a nerve root or spinal nerve extend proximally to involve the spinal cord, then myelopathy may be the only clinical sign. Lymphoma affecting the peripheral nervous system may be indistinguishable from nerve sheath tumor, based on clinical signs.

Diagnostic Techniques and Workup

A neoplasm affecting a peripheral nerve should be suspected when a complete physical and neurologic examination reveals the signs described in the previous section. Single-limb paresis and muscle atrophy in association with pain on manipulation of the affected limb are the most commonly recognized signs of a plexus neoplasm.

Electrophysiology

Electrophysiologic testing may define the location and extent of peripheral nerve involvement. Electromyography (EMG) may distinguish between atrophy resulting from either disuse or denervation or may
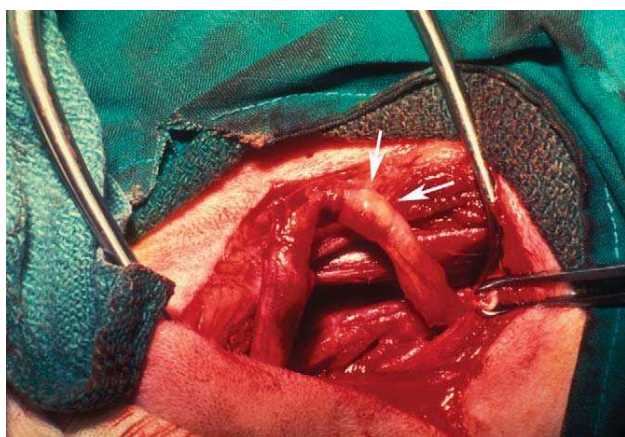
confirm the involvement of muscles not yet atrophied. Determinations of motor and sensory nerve conduction velocity may also provide information regarding nerve involvement. Evoked spinal cord potentials in combination with these determinations may be used to provide functional evidence of sensory nerve fiber connections with the spinal cord.[226,228]

Mapping

Mapping of atrophied muscles and regions of decreased cutaneous sensation in an affected limb may help to localize a peripheral nerve sheath neoplasm to a specific sensory or motor nerve, to a plexus, or to a group of spinal nerves or nerve roots.[226]

Radiography

Plain radiographs of the vertebral column, CSF analysis, and myelography are essential in cases in which secondary spinal cord involvement is suspected. The most frequently observed plain radiographic abnormality seen with a nerve sheath neoplasm is the widening of an intervertebral foramen or remodeling around an enlarged nerve root,[204] although plain radiographs frequently are normal.[225] Lymphoma seldom results in alterations visible on plain radiographs. Myelography is essential when neoplasia of spinal nerves or nerve roots is suspected, in order to detect invasion of the vertebral canal that may not be clinically apparent. At the time a myelogram is done, CSF collection and analysis may be completed; however, CSF analysis rarely aids in a more definitive diagnosis. The most commonly observed CSF alteration is an elevation of protein in the absence of a cellular response.

Surgical exploration for the biopsy of a cranial or peripheral nerve, nerve root, or plexus is essential for diagnosis. The use of CT and MRI to confirm the location and extent of a neoplasm prior to biopsy may be helpful in some cases.[229,230] In many dogs, surgical exploration may be the only method that can confirm the presence of a neoplasm.

Therapy

Presently, the treatment of peripheral nerve sheath tumors is limited to surgical excision.[19,206] Early detection and diagnosis of these tumors allow complete excision in only a few, unusual cases.[208,231] In the majority of animals, complete excision is impossible because of the proximal extension of the tumor into the vertebral canal or spinal cord.[232] Because a nerve sheath tumor generally involves several spinal nerves or nerve roots, the excision of spinal nerves as far proximally as possible, either at the level of the intervertebral foramen or at the level of the spinal cord following laminectomy and durotomy, is most commonly accompanied by amputation of the affected limb.[208] Postoperative irradiation

may be attempted but has not yet been adequately investigated. Chemotherapy for nerve sheath neoplasms may be of palliative benefit. A combination of vincristine, doxorubicin, and cyclophosphamide has been recommended.[41] Lymphoma of the peripheral nerves is treated in the same way as lymphoma is treated in other locations.

Prognosis

The overall prognosis for dogs with peripheral nerve sheath tumors is poor, and the recurrence rate after surgical treatment is high.[206] Dogs with nerve sheath tumors affecting a nerve peripheral to a plexus have a better prognosis than dogs with tumors affecting a plexus or invading the vertebral canal. Survival following surgical excision of schwannomas has been reported to range from 2 months to 2 years.[19,204] In one report,[233] a dog survived more than 3 years following the excision of a solitary schwannoma of the sixth cervical spinal nerve. Postsurgical metastases to the lung have been reported.[19,208] The high rate of local recurrence following surgical excision of a peripheral nerve sheath neoplasm may reflect the proximal extension of a tumor within a nerve trunk. Grossly visible margins of a tumor at the time of operative resection may not accurately demarcate the extent of tumor invasion.

Comparative Aspects

The types of nerve sheath tumors of dogs are similar to those of humans.[215] The controversy concerning classification of peripheral nerve sheath tumors in humans is similar to that discussed for dogs.[215,234] The goals of treatment for a benign peripheral nerve sheath neoplasm in humans are tumor resection and the preservation of nerve function. Most benign schwannomas in humans may be totally removed without nerve damage, and recurrence is rare.[234] Malignant nerve sheath neoplasms in humans have a high tendency to recur after local excision. A recurrence rate of over 40% following local excision has been reported. Radical excision, often in combination with amputation, is therefore recommended for the treatment of malignant nerve sheath tumors. Although the benefit of postoperative irradiation has not been proven, it has been recommended for humans.

Multiple cutaneous and peripheral neurofibromas are the hallmarks of von Recklinghausen's neurofibromatosis.[234] The peripheral nerve tumors associated with this disease may be benign or malignant in behavior, although a tendency exists toward malignant transformation. The similarity between this disease and neurofibromatosis in cattle and dogs has been noted; however, the disease as it occurs in humans has not been confirmed in dogs.[207]

NEUROLOGIC COMPLICATIONS OF SYSTEMIC CANCER

The incidence of neurologic complications of systemic cancer in dogs and cats is unknown; however, direct and indirect effects on the nervous system of cancer originating outside the nervous system are known to occur.[235-241]

More than 20% of humans with systemic cancer develop neurologic symptoms either as direct or indirect effects of their underlying disease (Box 29-2).[242,243] Metastasis to the brain is the most common complication of systemic cancer. Autopsy studies confirm that 25% of patients who die from cancer have intracranial metastases at the time of death. Metastasis to the spinal cord and nerve roots is the second most common neurologic complication of systemic cancer; it occurs in 5% to 10% of human cancer patients.[242]

Nonmetastatic nervous system complications of systemic cancer in humans occur less commonly than metastatic disease. Frequently, nonmetastatic complications develop acutely. They are often fully reversible, but they may be fatal if they are not recognized and treated.[242] Metabolic encephalopathy is a common nonmetastatic complication. It is a behavioral change that results from the failure of cerebral metabolism because of a systemic illness such as electrolyte imbalance, hepatic or renal failure, hypoxia, or sepsis.

Cancer patients may be susceptible to CNS infections because of altered immune mechanisms or neutropenia. Neutropenia is common in patients with hematogenous malignancies, in those with bone marrow depletion caused by a metastatic tumor, and in those receiving chemotherapy.

A high incidence of thrombocytopenia or coagulation deficits results in the increased risk of cerebrovascular complications. Embolic infarction may occur secondary to endocarditis or sepsis.

Neurologic complications may also result from cancer treatment.[242] Although permanent damage to the nervous system from irradiation is rare, it is known to occur. Several chemotherapeutic agents (e.g., methotrexate, cisplatin, vinca alkaloids, fluorouracil) may produce neurotoxicity in the central or peripheral nervous system, either directly or indirectly by toxicity to other organs such as the liver or kidney.[244]

Paraneoplastic syndromes or "remote effects" (see Chapter 5) are nervous system abnormalities that occur in patients with malignant systemic tumors but that are not caused by metastatic invasion of the nervous system or by any identifiable effect of cancer on the nervous system, such as infection or chemotherapy.[241,243,245,246] In about 50% of cases, nervous system symptoms precede the diagnosis of cancer. The cause and pathogenesis of paraneoplastic syndromes are not known, although possible mechanisms include autoimmune reactions, viral

infections, toxins secreted by a tumor, and nutritional deprivation. Commonly recognized paraneoplastic effects are sensorimotor polyneuropathy, myasthenia gravis (associated with thymoma), and polymyositis.[240]

Box 29-2	**Neurologic Complications of Systemic Cancer**

I. Metastasis to the Nervous System
   A. Intracranial neoplasia
   B. Spinal neoplasia
   C. Leptomeningeal neoplasia
   D. Cranial/peripheral nerve neoplasia
II. Nonmetastatic Neurologic Complications
   A. Metabolic encephalopathy
   B. Central nervous system infections
   C. Cerebrovascular disorders
   D. Adverse effects of treatment
   E. Paraneoplastic effects

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T umors of the eye, orbit, or adnexa can have devastating consequences for an animal's vision, appearance, and comfort, and they may be harbingers of potentially life-threatening disease elsewhere in the body. By virtue of their location, even benign ocular tumors may cause blindness and loss of the eye. Although these tumors reportedly affected only 0.87% of all dogs and 0.34% of all cats recorded in the Veterinary Medical Data Base (VMDB) over a 10-year period, their actual frequency is undoubtedly greater because many presumably benign ocular tumors are not histologically examined. This chapter describes the more common ocular tumors in small animals.

Tumors of the Eyelids, Third Eyelid, and Ocular Surface

Incidence and risk factors

Ocular papillomas tend to occur in young dogs and are believed to have a papovavirus etiology. Canine juvenile histiocytomas affect the eyelid skin of young to middle-aged dogs,[1,2] whereas benign adenomas and melanomas of the haired skin or eyelid margin tend to affect old dogs.[1,2] One study cites boxers, collies, Weimaraners, cocker spaniels, and springer spaniels at greater risk for eyelid neoplasia than the general hospital population,[1] and another suggests that beagles, Siberian huskies, and English setters are at greater risk than mixed-breed dogs.[2]

Squamous cell carcinoma (SCC) comprises up to two thirds of feline eyelid and third-eyelid tumors[3] and has a predilection for the lower eyelid and medial canthus of white cats (Figure 30-1). Ocular SCC is less frequent in dogs, but in both cats and dogs increased exposure to solar radiation, lack of adnexal pigmentation, and possibly chronic ocular surface irritation are believed to be predisposing factors.[1,4,5] Older (mean = 11 years), female, Weimaraner, and possibly German shepherd/large-breed dogs may be predisposed to conjunctival melanomas.[6]

Melanomas also have a propensity for the nictitating membrane and superior palpebral conjunctiva.[6,7] Corneal tumors have a predilection for the limbus.

Pathology and natural behavior

Sebaceous or meibomian gland adenomas and epitheliomas, papillomas, and melanomas constitute more than 80% of canine eyelid neoplasms, and a substantial majority (75% to 90%) of these tumors are histologically benign.[1,2] Even histologically malignant eyelid tumors in dogs rarely metastasize, although they are more likely to be locally invasive and recur following surgery.[1,2] In contrast, most feline eyelid and ocular surface tumors are malignant.

Viral papillomas tend to be well demarcated and superficial, minimally altering deeper tissues. Surgical manipulation has occasionally been associated with dispersal of papillomas throughout the ocular surface.[8,9] Papillomas and histiocytomas often spontaneously resolve in young dogs, although they may persist in the older dog. SCC may also develop superficially, but, following malignant transformation, a preinvasive actinic plaque can invade deeper tissues. Late in the course of the disease, SCC may spread to regional lymph nodes and, uncommonly, distantly metastasize. SCC of the third eyelid may more readily invade the orbit than corneal or eyelid SCC.

Adenocarcinomas of the gland of the third eyelid are variable in morphology and often show moderate infiltrative growth.[10] They may mimic prolapse of the gland of the nictitans ("cherry eye") by appearing as localized, firm, smooth, pink swellings on the posterior surface of the nictitans, but a key differentiating feature is their occurrence in much older dogs (10 to 16 years). Although excision of the grossly visible tumor may initially appear adequate, recurrence is common if the entire gland is not removed, and metastasis, especially to the regional lymph nodes and orbit, is possible.[10,11]

Figure 30-1

Squamous cell carcinoma has a predilection for the medial canthus of elderly white cats. *(Photograph courtesy of Dr. Christopher J. Murphy.)*

The natural behavior of conjunctival vascular, melanocytic, and mast cell tumors is poorly understood, in part because they are uncommon. Conjunctival hemangiomas and hemangiosarcomas tend to remain relatively superficial but may recur following simple excision.[12-14] Hemangiosarcomas may exhibit a more aggressive course, and a primary ocular hemangiosarcoma must be differentiated from a metastatic lesion. Metastasis of primary conjunctival vascular tumors, even when classified as hemangiosarcomas, however, appears to be rare.[12-14] Feline conjunctival melanomas originate on the bulbar conjunctiva and invade the eyelid.[15] Similarly, canine conjunctival melanomas have been reported to recur locally following surgical excision in 55% of cases, and at least 17% of the dogs experienced orbital invasion or spread to the regional lymph nodes or lungs.[6] Melanomas originating from the palpebral conjunctiva may have greater metastatic potential.[6] Mitotic index, cell type, and degree of pigmentation are not useful predictors of malignancy for canine conjunctival melanomas.[6] In general, subconjunctival mast cell tumors appear to have a relatively benign course in dogs.[16]

History and clinical signs

Vascular tumors are often focal, raised, soft, red masses with visible feeder vessels arising from the surface of the conjunctiva or third eyelid.[12-14] SCC of the eyelid, third eyelid, or ocular surface may appear as a focally thickened, roughened, usually pink to red lesion in older animals or, more commonly, as an ulcerated lesion with a protracted course. In contrast, papillomas in young dogs appear verrucous and usually progress rapidly over weeks to a few months. Fibrous histiocytomas

(also called nodular granulomatous episcleritis in some locations) are rapidly growing inflammatory lesions that appear as nonulcerated, smooth, raised, pink nodules originating from the limbus, sclera, third eyelid, or eyelid. The degree of pigmentation, however, does not reliably predict the histologic nature of ocular surface tumors, especially those involving the eyelids.[1]

In addition to a mass lesion, other clinical signs of eyelid or ocular surface tumors may include epiphora, conjunctival vascular injection, mucopurulent ocular discharge, protrusion of the third eyelid, conjunctival/corneal roughening or ulceration, and corneal neovascularization or pigmentation. Occasionally, palpebral conjunctival masses protrude only when their bulk no longer can be accommodated by the space between the eyelid and globe, and very advanced tumors may create exophthalmia or enophthalmia if the orbit is invaded. Large tumors and sebaceous adenomas often have a substantial inflammatory component and may be secondarily infected.

Diagnostic techniques and workup

In addition to fluorescein staining and examination of the ocular surface with a cobalt filter or blacklight, the extent of involvement of the bulbar and palpebral conjunctiva should be determined by everting the eyelid (and third eyelid if affected). Careful palpation of the lesion by inserting a lubricated finger in the conjunctival cul-de-sac can be invaluable for determining the full extent of the tumor and whether bony involvement has occurred or not. Nasolacrimal lavage and possibly positive contrast dacryocystorhinography may help characterize medial canthal masses. In general, small eyelid and ocular surface tumors are best diagnosed and treated by excisional biopsy. Fine-needle aspirates or incisional biopsies of larger tumors aid in determining prognosis and planning definitive therapy. Occasionally, orbital ultrasound, skull radiographs, computed tomography (CT), magnetic resonance imaging (MR), regional lymph node cytology, and thoracic radiographs are required to localize or clinically stage potentially malignant tumors such as SCC, mast cell tumors, adenocarcinomas of the third eyelid, and conjunctival melanomas.

Therapy

Specific therapy varies with the type of tumor; its location, size, and extent; whether or not the eye still has useful vision; the animal's expected life span; the degree of discomfort the mass is creating; and the owner's financial limitations. All eyelid tumors, whether benign or malignant, have the potential to affect vision or ocular comfort. Indications for tumor removal include any eyelid tumor in a cat, rapid growth, ocular surface irritation, impaired eyelid function, owner concern, or an unappealing appearance. In young dogs, observation

of nonirritating papillomas or histiocytomas may be appropriate, as spontaneous regression is common.

Tumors involving less than one fourth to one third of the length of the eyelid are best treated by a V-plasty (wedge) or four-sided excision.[17] The latter technique affords superior apposition of the eyelid margins and wound stability, especially in tumors approaching the one-fourth to one-third limit, because the initial incision is made perpendicular to the eyelid margin rather than obliquely. In general, only one fourth of the feline eyelid can be removed with these techniques. Antibiotic or anti-inflammatory therapy may reduce the size of large tumors that are infected or inflamed so that a wedge or four-sided excision becomes possible. Electrosurgical excision should be avoided, since it may result in substantial scarring of the eyelids. CO_2 laser ablation may be appropriate for some tumors.

Tumors greater than one fourth to one third of the eyelid typically require more advanced reconstructive blepharoplasty or utilization of other therapeutic modalities. Some tumors may be responsive to systemic chemotherapy (e.g., lymphoma, mast cell tumors), local infiltration with chemotherapeutic agents such as cisplatin (SCC),[18] or local radiation therapy (SCC). In some cases, these modalities will completely eliminate the tumor or cytoreduce it to the point where a less extensive surgical procedure can be performed. Reconstructive blepharoplasty, however, is the procedure of choice if surgical cure is a possibility and these other modalities have failed or are unlikely to substantially impact the tumor.

Cryosurgery is an attractive alternative to extensive blepharoplasty and has been reported to be effective in several canine eyelid tumor types (see Chapter 15A).[2,19] It is quick, less technically demanding than reconstructive blepharoplasty, and usually preserves the naso-lacrimal puncta and canaliculus. In many old or debilitated patients, cryosurgery can be accomplished with only sedation or local/topical anesthesia. Following pretreatment with dexamethasone (0.1 mg/kg IV), the mass is isolated with a chalazion forceps (if possible) and debulked flush with the lid margin. Using liquid nitrogen and a closed probe that approximates the diameter of the mass as much as possible, a double freeze-thaw is performed so that the iceball extends 3 to 5 mm beyond the visible margins of the mass. Iceballs should overlap in large tumors. Freezing may be repeated a second or third time if the mass does not completely regress following the first session. Substantial postoperative swelling and usually transient depigmentation of the frozen tissue are to be expected.

Tumors involving the conjunctiva and third eyelid (especially conjunctival hemangiosarcomas, melanomas, and nictitans adenocarcinomas) are most effectively treated by wide surgical excision—in some cases to the point of exenterating the orbit. If the globe is to be spared, however, excision of the entire nictitans should not be taken lightly because undesirable sequelae such as ocular drying and chronic keratitis frequently result. Bulbar conjunctival tumors move freely and, if small, are generally amenable to excision under only topical anesthesia and perhaps sedation. Cryosurgery may permit the nictitans to be spared in the cases of papillomas and early SCC, or it can be used as an adjunct to excision in advanced canine conjunctival melanomas and SCC.[6]

Superficial keratectomy/sclerectomy is preferred for many corneal and scleral tumors, although some tumors require a full-thickness resection of the cornea or sclera. In the latter case, corneal or scleral allografts or autologous tissue grafts should be used to maintain ocular structural integrity. Limbal SCC and epibulbar melanoma may also be amenable to cryosurgery, although the iceball should be carefully monitored to avoid unnecessary freezing of intraocular structures.

Prognosis

The prognosis for most canine primary eyelid tumors is excellent, whether treated by excision or cryosurgery. Metastasis is rare, even in histologically malignant primary lid tumors, and recurrence rates are low (approximately 10% to 15%).[2] New primary eyelid tumors are not uncommon and must be distinguished from recurrence. Because most eyelid tumors in cats are malignant, the prognosis is not as good as that for dogs, but it is unclear how prognosis correlates with the histologic features. Conjunctival melanomas and nictitans adenocarcinomas frequently recur following partial excision of the nictitans, even if all of the clinically visible tumor was removed.[6] Conjunctival hemangiosarcomas appear to have a good prognosis, since total excision may be curative, although recurrence and loss of the eye is still possible.[13,14]

LIMBAL (EPIBULBAR) MELANOMAS

Limbal melanomas in dogs and cats are typically benign, slightly raised, heavily pigmented masses originating from melanocytes in the sclera or subconjunctival connective tissue (Figure 30-2).[20-24] The vast majority of these slow-growing tumors originate in the superior limbal region, suggesting that exposure to solar radiation may be a risk factor.[12] Affected dogs average 5 to 6 years old (cats 8+ years), and a female and German shepherd breed predilection has been inconsistently reported.[20-23] Confirmed metastasis has not been reported in dogs or cats, and mitotic figures are rarely encountered; although in one study, two of four cats also had FeLV-associated lymphoma or leukemia, and a third cat had a second intraocular pigmented mass unassociated with the limbal tumor.[23] Lightly pigmented spindle cells capable of division are seen histologically, but the dominant cell is presumably a hypermature spindle cell that is large, round, pigment laden, and benign.[20] These masses are often only incidentally noted
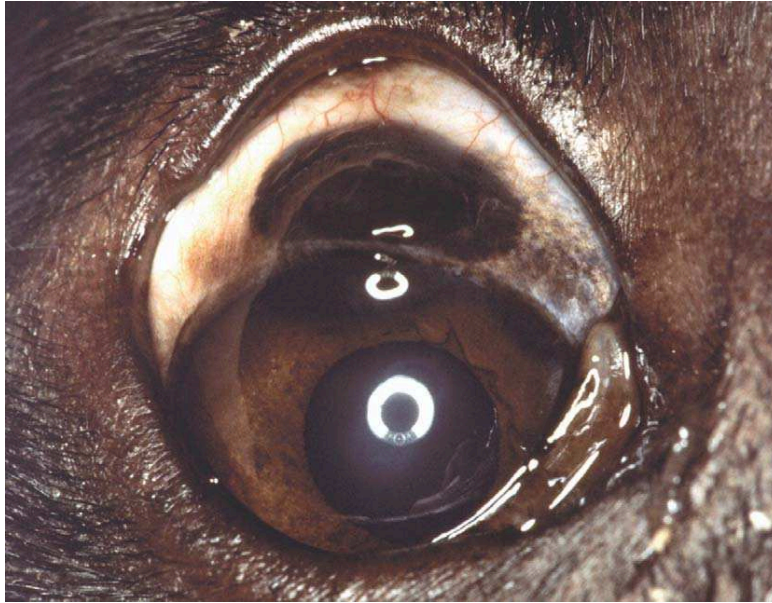
Figure 30-2

Epibulbar melanomas typically originate from the superior limbal region of the globe.

and the clinical signs are typically minimal, although local corneal invasion, epiphora, and mild conjunctival irritation may be seen.[21-22] Differential diagnoses include conjunctival melanoma, invasive uveal melanoma, metastatic melanoma, and staphyloma or coloboma. Gonioscopy aids in differentiating invasive intraocular tumors from limbal melanomas.

Therapy should be considered if the tumor has invaded the eye or if growth is rapid. Given its benign nature and usually slow growth rate (imperceptible growth over 18 months has been described), observation alone may be appropriate in older dogs. If intervention is required, lamellar keratectomy/sclerectomy with graft placement is often curative. Beta-irradiation and cryosurgery have been used as adjuncts to surgery. Cryosurgery alone or, more recently, laser photocoagulation[24] has also been described as effective means of treatment. Regrowth following local surgical excision occurs in approximately 30% of patients, but 2 to 3 years may pass before the anterior chamber is invaded and enucleation is required.[20-22] Enucleation is curative and is indicated if painful intraocular disease is present.[20]

PRIMARY OCULAR TUMORS

Canine Anterior Uveal Melanomas

Incidence and risk factors

In the Armed Forces Institute of Pathology collection of tumors from the canine eye, orbit, and adnexa, intraocular melanomas constituted 12%, other primary intraocular tumors 14%, and metastatic intraocular neoplasms 9%.[25] Any age is at risk, but most affected dogs are older than 7 years, and breed or sex predilections are inconsistent.[20] Ocular melanomas have been induced experimentally by exposing dogs to radium and by intraocular injections of feline sarcoma virus in kittens.[26]

Pathology and natural behavior

The majority of canine intraocular melanomas are benign, and arise from the iris or ciliary body (Figure 30-3).[20,21,27] Several classification schemes based on cell type and growth pattern have been proposed, generally with little correlation to clinical outcome or natural behavior. The most clinically useful scheme classifies these tumors simply as melanocytoma (benign) and melanoma (potentially malignant).[20] Benign tumors have <2 mitotic figures/10 high-power fields (mitotic index), and malignant tumors demonstrate nuclear pleomorphism and a mitotic index of at least 4 and often >30. Destruction of the eye is not by itself sufficient for a diagnosis of malignancy.[20] The overall rate of metastasis of intraocular melanomas is approximately 4%[27] and usually occurs via the hematogenous route. Local spread along ocular vessels and nerves or via direct penetration of the sclera or cornea also occurs. Benign tumors tend to be more darkly pigmented than malignant tumors.[20]

Circumscribed, nevus-like pigmented iridal growths have been described in young dogs (7 months to 2 years old).[21] The natural history of these lesions is variable, as enlargement may not occur over several years.[21,25]
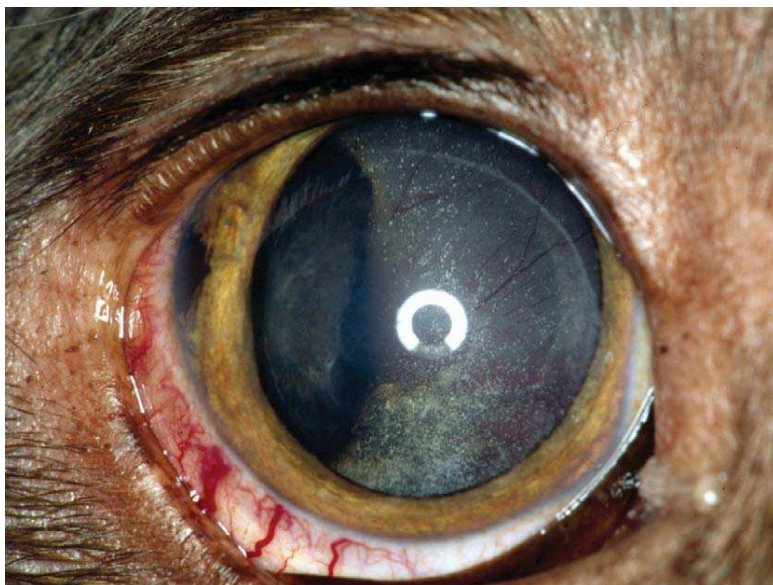
Figure 30-3

Most anterior uveal melanomas originate from the iris or ciliary body and are benign.

Some, however, are capable of rapid growth, but to date all are clinically and histologically benign.

History and clinical signs

Common presentations of intraocular tumors include a visible intraocular or scleral mass, glaucoma, hyphema, anterior uveitis, extrabulbar spread, or as an incidental finding during ophthalmic examination.[20,27] Because glaucoma or hyphema are often the only overtly visible clinical signs,[20,27] intraocular neoplasia should always be considered in animals with hyphema, glaucoma, or both, when there is no history of trauma or coagulopathy. Small masses frequently create few symptoms other than pupillary distortion. Pigmentation varies and is not a reliable indicator of tumor type.

Diagnostic techniques and workup

Usually the clinical or ultrasonographic appearance (if the media are opaque) strongly suggests intraocular neoplasia, although it may be difficult to arrive at a definitive diagnosis without invading the eye or removing it. Because most anterior uveal brown or black masses are cystic and not neoplastic, transillumination should be attempted before more invasive procedures. Uveal cysts typically permit bright light to pass through them, are roughly spherical, and may be attached to the ciliary body or free-floating in the anterior chamber. Once suspected, most primary canine intraocular tumors are observed for progression, although occasionally fine-needle aspiration (with its risks of inflammation, infection, and hemorrhage) or attempts at intraocular resection or enucleation are used for diagnostic purposes. The possibility of metastasis from another primary site (i.e., oral cavity or nail bed) to the eye, or from the eye to other organs should be eliminated.

Therapy

Canine primary intraocular tumors are generally not treated. Enucleation is advised if there is concern about malignancy or if complications such as intractable uveitis or secondary glaucoma occur.[28] The low risk of metastasis and unproven efficacy of enucleation at preventing metastasis in the few malignant tumors that have been reported, however, make it difficult to automatically recommend enucleation of normotensive, noninflamed, visual eyes.[20] Isolated primary masses involving only the iris or a portion of the ciliary body may be amenable to local resection by sector iridectomy/cyclectomy in order to preserve the eye and vision.[21,25] These intraocular procedures, however, require an accomplished ophthalmic surgeon and often have unsatisfactory long-term results. Recently, trans-scleral and transcorneal Nd:YAG or diode laser therapy has induced remission in some small to moderate-size primary intraocular tumors.[28,29] Although the results varied, perhaps because these tumors varied histologically in nature, this relatively new method holds promise for the palliation or potential cure of a number of intraocular tumor types while also preserving vision. Metastasis was not observed following this procedure, although this obviously remains a risk when the tumor is malignant.[28]

Diffuse iris melanomas may first appear as multifocal to diffuse pigmentary changes, as seen in the coalescing darker regions of the iris in this cat.

Prognosis

Although the data in most studies are heavily censored, the prognosis for histologically benign melanomas appears to be excellent. Enucleation is curative, but attempts at local excision or laser photoablation may be only palliative, especially if the ciliary body or trabecular meshwork is involved. The presence of black, nonsolid material within the orbit following the enucleation of benign melanomas with scleral invasion apparently does not affect prognosis, because these cells and pigment debris appear incapable of continued growth.[21] In one study, approximately 25% of histologically malignant melanomas demonstrated metastasis, typically within 3 months of enucleation, and most dogs with metastasis are euthanatized within 6 months of enucleation.[20] In a larger study, dogs with tumors classified as malignant were reported to have only a somewhat decreased survival time compared to dogs with melanocytoma and dogs from a control population.[30]

Choroidal Melanomas

Choroidal melanomas are rare intraocular melanocytic tumors that make up only 4% of canine uveal melanomas, with no clear breed or sex predisposition.[31] Middle-aged (6 to 7 years), medium- to large-breed dogs predominate.[31] Generally, these tumors are well delineated, raised subretinal pigmented masses with tapering margins, bulging centers, and a propensity for the peripapillary region and optic nerve.[31,32] Some of these tumors remain virtually static for many years, whereas others exhibit infiltration into the overlying retina, through the sclera, up the optic nerve, and into the orbit.[31] Nuclear anaplasia is minimal and generally mitotic figures are absent.[32] Despite these benign cytologic features, metastasis has been described in 1 dog 21 months after exenteration, and follow-up in most studies is incomplete.[33] In general, however, these tumors appear to be benign in the vast majority of dogs. Most dogs with tumors involving a limited portion of the choroid are asymptomatic, and the mass is noted incidentally on ophthalmoscopy. Larger tumors frequently present with chronic uveitis, secondary glaucoma, retinal detachment, intraocular hemorrhage, or blindness.[31,32] Ocular ultrasonography may demonstrate mass lesions if anterior segment changes or retinal detachment obscures an underlying mass. Therapy usually consists of enucleation once progression has been documented or if the eye is painful. Diode laser ablation may offer an alternative to enucleation if the lesion is small and does not involve the optic nerve. Optic nerve or scleral invasion may warrant a more cautious prognosis.

Feline Primary Intraocular Melanomas

Incidence and risk factors

Anterior uveal melanomas are suggested to be the most common primary intraocular tumor in cats (Figure 30-4).[34] No breed or sex predisposition is evident, and most cats are more than 9 years of age at

the time of diagnosis,[34,35] although the prodromal period for many of these tumors may be quite long.

Pathology and natural behavior

In the malignant form of uveal melanoma, the rate of metastasis (frequently to the liver and lungs) has been reported to vary from 55% to 66% or higher.[34-37] Iridal hyperpigmentation, however, frequently takes months to years to progress to the extent to which the eye must be enucleated, and an additional 1 to 3 years after enucleation are required before metastatic disease may become evident.[34,35,37,38] No single morphologic feature predicts outcome, but metastasis has been linked to a greater mitotic index, larger tumors, and extension through the iris into the ciliary body stroma and involvement of the scleral venous plexus.[34,35]

A poorly characterized, but apparently more common, benign form of uveal melanoma also appears to exist, as most ophthalmologists have noted unilateral or occasionally bilateral progressive iridal pigmentary changes (especially in older orange cats) that apparently do not lead to metastatic disease over many years to a decade or more. It is possible in some cats that these initially benign-appearing accumulations of small angular pigmented cells on the anterior iridal surface undergo transformation to the larger, rounded cells typical of the potentially malignant diffuse iris melanoma. Of concern to the clinician waiting to document progression before advising treatment, however, is that malignant transformation is not readily observable clinically and that these cells, once transformed, appear to be capable of quickly dropping off into the anterior chamber and entering the drainage apparatus and vasculature.[35]

History, clinical signs, diagnostic techniques, and workup

Slowly progressive, diffuse iridal hyperpigmentation is the most common clinical sign, although occasionally a pigmented iridal nodule or amelanotic mass is seen. Secondary glaucoma may occur, and the diffuse form may be mistaken for chronic anterior uveitis with iridal hyperpigmentation.

The diagnosis of melanoma, generally made clinically, requires demonstration of progression and iridal thickening or irregularity of the iris surface or pupil. The prognostic and diagnostic value of fine-needle aspirates of the iridal surface or iridal biopsies is unclear and worthy of further study.

Therapy

The treatment of feline uveal melanomas is controversial. Ideally enucleation would be delayed until just *prior* to malignant transformation, invasion into other ocular structures, secondary glaucoma, or metastasis. Such precise timing, however, is seldom attainable in a clinical setting and enucleation is commonly performed if iridal pigment changes have been demonstrated to progressively increase to the point that virtually the entire iridal surface is involved, pigmented cells are present in the trabecular meshwork, the pupil is distorted (indicating iridal invasion), ciliary body or scleral invasion is threatened, if uveitis is present, or glaucoma is impending. Although it would seem logical that early enucleation would optimize survival, this is unproven. In one study, enucleation has been shown to markedly enhance the rate of metastasis in cats with feline sarcoma virus–induced uveal melanomas.[26] The applicability of this experimental model to spontaneous disease, however, is unclear. Some ophthalmologists have attempted to ablate small, focal, hyperpigmented foci on the iris of cats with a diode laser, thereby preserving vision and the eye. The long-term success rate and side effects of this procedure, however, are not known. Finally, most slowly progressing lesions are simply monitored, ideally by comparison to baseline photographs. This option is particularly suitable for older cats with other diseases that limit their expected life span. In many cats, progression may be so slow, allowing the patient to be followed for many years to a decade or more without apparent metastasis.

Prognosis

One study correlates the metastatic potential of feline uveal melanomas with the extent of ocular involvement seen histologically.[35] Because the tumor is relatively slow growing, however, the period until metastatic disease becomes apparent may be measured in years, and even then, substantial additional time may be required before the metastasis is life threatening. Cats with tumors confined to iris stroma and trabecular meshwork at the time of enucleation have survival times comparable to those of age-matched controls. Enucleation after the tumor has invaded into the ciliary body but not the sclera warrants a poorer prognosis, but median survival time is still approximately 5 years.[35] Enucleation after the tumor has invaded into the ciliary body and the sclera merits an even poorer prognosis, with a median survival time of approximately 1.5 years.[35] The median survival time also decreases if secondary glaucoma has occurred.[35]

Feline Primary Ocular Sarcomas

Sarcomas following ocular trauma, although uncommon, may be second only to melanomas in frequency as a primary ocular tumor of cats.[39-42] Cats 7 to 15 years of age are most commonly affected, and the latency period following trauma averages 5 years.[40] Damage to the lens and chronic uveitis may be risk factors.[39-41] Although the cell of origin and the role of feline sarcoma virus is unclear, it is likely that chronic inflammation may

eventually lead to neoplastic transformation of a pluripotent cell.[39-41] These tumors, often within the same eye, exhibit a spectrum of changes ranging from granulation tissue, to fibrosarcoma, osteosarcoma, and anaplastic spindle cell sarcoma.[40,41] A subset of tumors have a pure round cell population.[43] They tend to circumferentially line the choroid and quickly infiltrate the retina and optic nerve (Figure 30-5).[40,41] White or pinkish discoloration of the affected eye or change in the shape or consistency of the globe are the most common presenting signs. Skull radiographs may demonstrate bone involvement or metallic foreign bodies.[42]

Because this tumor is uncommon, many ophthalmologists will not remove a comfortable phthisical feline eye unless it changes appearance. The advanced stage at which many of these tumors are first identified and the propensity for early optic nerve involvement, however, indicate that enucleation at this point may be only palliative and not prolong life. This has led some authors to advocate prophylactic enucleation of phthisical feline eyes or of feline eyes that are blind and have been severely traumatized or are chronically inflamed.[39,42] As much of the optic nerve as possible should be removed during enucleation for ocular sarcoma so that the extent of infiltrative disease and prognosis may be accurately determined. There is reason to believe that the prognosis is considerably better if enucleation is performed before the tumor invades the optic nerve or extends beyond the sclera.[44] To date, there have been no reports of treatment by radiation or chemotherapy.

Extraocular extension is common, as is reoccurrence following orbital exenteration.[39,42] Continued growth up the remainder of the optic nerve into the chiasm and brain with vision loss or other neurologic signs, involvement of regional lymph nodes, and distant metastasis has been reported.[39,40] The majority of animals die from local invasion and recurrence, typically within several months of enucleation.[39,42]

Spindle Cell Tumors of Blue-Eyed Dogs

Dogs with blue, or partially blue irides appear to be at risk of developing a spindle cell sarcoma in the anterior uvea.[45] These tumors always involve the iris, but they often extend into the ciliary body and even into the vitreous. Breeds that commonly have blue irides appear to be more likely to develop an iridal spindle cell sarcoma, but any dog with any blue in its iris appears to be at risk. Although the origin of these tumors is unclear, they have several features that suggest peripheral nerve differentiation. In a case series involving 11 dogs, more than half of the tumors were not clinically recognized, and the diagnosis of neoplasia was not made until histopathology was performed. Metastatic disease has not been seen; however, local recurrence within the scleral shell was observed in a dog that had been treated by evisceration and placement of an intrascleral prosthesis.[45]

Ciliary Body Epithelial Tumors

Primary ciliary body tumors (ciliary body adenomas and adenocarcinomas and, less commonly, medulloepitheliomas and astrocytomas) are infrequent in dogs and rare in cats.[46] The two main histologic forms that have been described are papillary (57% of cases in one study) and solid tumors (43%).[46] In the authors' experience, these tumors often appear nonpigmented clinically, but histologically at least some pigmented cells are present in approximately one half of cases. Pigmented tumors of the ciliary body may be grossly indistinguishable from anterior uveal melanomas. Middle-aged to older dogs are the most commonly affected and golden and Labrador retrievers may be predisposed, for they make up 27% of dogs with iridociliary epithelial tumors in one survey.[46] Most of these tumors appear to be benign, fairly well delineated, sometimes pedunculated, slow-growing masses that originate in the pars plicata of the ciliary body.[46,47] Although approximately 60% invade the uveal tract, only 21% invade the sclera.[46] Tumors that invade the sclera are typically classified as adenocarcinomas and

Figure 30-5

A primary ocular sarcoma secondary to ocular trauma in a cat. The tumor completely fills the anterior and posterior chamber and extends into the optic nerve.

have anaplastic features, but metastasis is uncommon and occurs late in the course of the disease, if at all.[46-49]

Clinical signs include a retro-iridal mass that may displace the iris or lens by expansive growth, and if the tumor is large, secondary glaucoma, ocular pain, and intraocular hemorrhage may be noted.[48] The diagnostic workup and differential diagnosis are similar to that of anterior uveal melanomas. Given the high frequency of ciliary body cysts in some predisposed breeds (especially the golden retriever), it is essential to differentiate ciliary body tumors from a benign cystic lesion prior to enucleation. Cystic lesions, which rarely require any intervention, are usually seen as lightly pigmented, ovoid, retro-iridal masses that can be shown to be hollow by transillumination. Early enucleation of ciliary body tumors has been recommended, although benign adenomas may remain static for years, making enucleation controversial for small tumors unassociated with secondary ocular disease. Local intraocular resection or laser photoablation may safeguard vision and ocular comfort.[28] Systemic administration of 5-fluorouracil has been described as an adjunct to local resection of ciliary body tumors, but the efficacy of this therapy is unknown.[49]

Secondary Uveal Neoplasms

Numerous malignant tumors, especially adenocarcinomas, have been reported to metastasize to the highly vascular uveal tract. Lymphoma is the most common secondary intraocular tumor in the dog and cat, and ocular lesions are present in approximately one third of dogs with the disease.[48,50-52] Common presentations include severe uveitis, glaucoma, retinal hemorrhages, hyphema, conjunctivitis, and keratitis characterized by corneal infiltrates, edema, vascularization, and intrastromal hemorrhage.[48,50-52] Exophthalmia resulting from orbital invasion by the tumor and vision loss due to optic nerve or central nervous system disease may also be present. Posterior segment lesions may include retinal vascular tortuosity, papilledema, multiple intraretinal hemorrhages, and retinal detachment. In one study, the life span of dogs with intraocular lymphoma was only 60% to 70% as long as dogs without ocular involvement when treated with cyclophosphamide, vincristine, and prednisolone (COP), or with doxorubincin.[51] Topical or systemic corticosteroid therapy or enucleation is palliative. (See Chapter 31 for the definitive therapy of lymphoma.) Ophthalmic disease may also be the presenting complaint in animals with multiple myeloma.[53]

Tumors of the Orbit and Optic Nerve

Incidence and risk factors
Risk factors other than middle to old age, possibly large breed dogs,[54] and possibly sex (female dogs, male cats)

have not been described.[54-56] Tumors involving the optic nerve are rare, although secondary invasion occurs in feline posttraumatic sarcomas and canine choroidal melanomas. Intraorbital meningiomas, the most common tumor of the optic nerve, constitute only 3% of all meningiomas in dogs.[57] Lobular adenomas of unspecified glandular origin have been reported to involve the orbit in dogs.[58]

Pathology and natural behavior
Orbital neoplasia may be primary (most common in dogs), secondary to extension of adjacent tumors into the orbit (most common in cats), or the result of distant metastasis. In cats and dogs, more than 90% of orbital tumors are malignant, and regional infiltration (including into the CNS) or distant metastasis is common.[54-56] At least 26 types of orbital tumors, roughly equally divided among connective tissue, bone, epithelial, and hemolymphatic origins, have been reported in dogs.[56] Osteosarcomas, mast cell tumors, reticulum cell sarcomas, fibrosarcomas, and neurofibrosarcomas are the most common canine primary orbital tumors.[56] More than two thirds of feline orbital tumors are epithelial in origin, with SCC being the most common,[55] but at least 15 other tumor types have been described in cats.

Canine orbital meningiomas exhibit unpredictable biologic behavior. Although they rarely distantly metastasize, they may be osteolytic and invade surrounding tissues, including the CNS.[57,59,60] Primary optic nerve tumors in dogs include neurofibrosarcoma, glioma, and meningioma.[57,58,60,61] Lobular orbital adenomas are made up of multiple friable lobules in the anterior orbit making complete surgical excision difficult.[59]

History and clinical signs
Slowly progressive exophthalmia, absent to minimal pain on opening the mouth, difficulty in retropulsing the eye, and deviation of the globe typify orbital neoplasia. Sudden erosion of nasal or sinus tumors into the orbit occasionally results in acute exophthalmia and substantial orbital pain. Enophthalmia may occur if the mass is anterior to the equator of the globe. Lobular adenomas may present as soft, raised, subconjunctival masses and create either enophthalmia or exophthalmia. Chronic epiphora secondary to obstruction of the nasolacrimal duct, exposure keratoconjunctivitis, palpable orbital masses following enucleation, or unexplained orbital pain also suggests orbital neoplasia.[55,56] Intraocular pressure measurement differentiates glaucomatous ocular enlargement from exophthalmia.

Optic nerve lesions may result in unilateral or bilateral blindness (the latter if the optic chiasm is affected); optic nerve head pallor, papilledema, or marked protrusion and congestion on ophthalmoscopy. A relatively mild degree of exophthalmia with vision loss suggests optic nerve neoplasia, because tumors of other orbital

tissues typically cause profound exophthalmos before visual loss. Tumors affecting the retrobulbar, intra-canalicular, or chiasmal portions of the optic nerve may not result in exophthalmia or a visible change in the optic nerve head.

Diagnostic techniques and workup

It is essential to differentiate non-neoplastic orbital inflammatory diseases (granulomas, cellulitis, abscesses, myositis of the extraocular and masticatory muscles) from neoplasia. Animals with inflammatory disease typically exhibit significant pain on opening the mouth. The location of an orbital mass can usually be determined by careful physical examination, including retropulsion of the globe, oral examination caudal to the last molar, and determination of the direction of malposition of the eye.

In addition to physical examination, cytology of regional lymph nodes, orbital ultrasound, and skull and thoracic radiographs should be performed. In one study of cats with orbital neoplasia, 59% had radiographic signs of orbital bone lesions, and 15% had evidence of metastasis on thoracic radiographs.[55] Computed tomography or magnetic resonance imaging offer superior depictions of the orbit and facilitate planning of either radiation or surgical therapy (Figure 30-6). Histologic characterization by fine-needle aspirates or needle core biopsies (performed via the mouth or through the orbital skin), with ultrasound guidance if necessary, help to lead to a definitive diagnosis. The globe, major orbital blood vessels, and optic nerve should be avoided. Because 50% of orbital tumors may have a nondiagnostic fine-needle aspirate, especially in cases of SCC,[55] exploratory orbitotomy via a number of approaches[62,63] or exenteration may be required to characterize the mass and resect it if possible. Cerebrospinal fluid taps may aid in distinguishing optic nerve neoplasia from optic neuritis.

Therapy

Primary orbital and optic nerve tumors that lack metastasis or regional lymph node involvement may be amenable to surgical excision. If bony involvement is not present, orbital exenteration by widely dissecting around the mass (stripping periorbita if necessary) is usually the preferred procedure, as the advanced stage of the tumor at the time of diagnosis typically makes it impossible to excise the mass completely and preserve a functional or comfortable eye. If periorbital bones are involved, radical "orbitectomy," which resects the affected orbital tissues and surrounding bones, should be considered.[64] When treating optic nerve tumors, as much of the ipsilateral optic nerve as possible should be removed in an attempt to obtain complete excision.[57]

If preservation of a comfortable eye and vision appears possible, a variety of orbitotomy techniques,
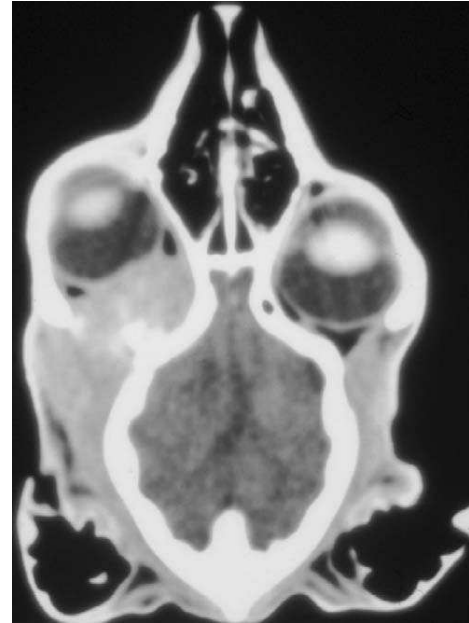
Figure 30-6

Computed tomography (dorsal view) provides detailed information about the location and extent of orbital tumors, as in this 17-year-old cat with orbital osteosarcoma.

ranging from small incisions through the eyelid or mouth to reflection of the zygomatic arch, have been described.[62,63] Postoperative complications are common and may include secondary enophthalmia with entropion and possibly diplopia (double vision). Surgical debulking can be palliative, and some dogs may survive a year or more with minimal therapy.

The role of chemotherapy and radiation therapy, either alone or as an adjunct to surgery, is yet to be defined in the treatment of orbital tumors, although chemotherapy for orbital lymphoma may be effective. Systemic corticosteroids may permit some patients with optic nerve meningioma to maintain vision for several weeks to months. Radiation therapy may be helpful in the case of nasal tumors with orbital extension,[55,56] in subtotally excised or recurrent meningiomas,[59] and in other select cases.

Prognosis

With conservative treatment the prognosis for most tumors involving the orbit and optic nerve is poor,[55,56] especially if there is bony involvement on skull radiographs. Recurrence at the primary site and involvement of adjacent or distant sites are common, often occurring within weeks to a few months. Even benign-appearing tumors such as lobular orbital adenomas and orbital meningiomas may be locally invasive and have a propensity for recurrence following wide excison.[56,58,60,64]

In one study, however, radical orbitectomy (with or without chemotherapy or radiation therapy) provided a local disease-free interval of more than 1 year in >50% of patients and a 70% survival rate for the first year.[64] In another study, the mean survival time for cats with orbital tumors treated by radiation therapy, chemotherapy, or surgery that included resection of affected orbital bones was only 4.3 months.[55] In a study of 23 dogs with orbital tumors, most of whom were treated by exenteration with or without adjunct therapy, only 3 survived 3 years or longer.[56] The majority of these animals died as a direct result of the tumor or were euthanatized at the time of diagnosis.[54-56]

OCULAR EFFECTS OF CANCER THERAPEUTIC MODALITIES

The ocular effect of external beam radiation therapy for nasal and periocular tumors can have a substantial impact on an animal's quality of life. Common complications include chronic keratoconjunctivitis, corneal ulceration, "dry eye," enophthalmia, entropion, cataracts, retinal hemorrhages, retinal detachments, and blindness (Figure 30-7).[65,66] Many of these conditions respond poorly to treatment, and vigorous attempts at prevention should be made to avoid chronic ocular pain and blindness. Intensity modulated radiation therapy, which uses conformal avoidance, has been shown
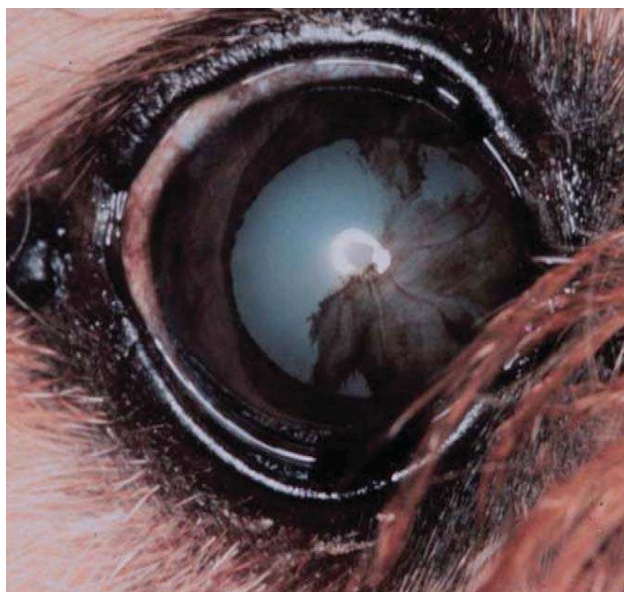
Figure 30-7

Radiation therapy for periocular tumors can cause significant ocular complications, such as keratoconjunctivitis and cataract formation, as in this dog.

to significantly decrease the ocular toxicity seen in dogs treated by radiation therapy for spontaneous sino-nasal tumors.[67] In humans, blurred vision, partial visual field defects, loss of color vision, and diplopia have been associated with several antineoplastic drugs.[68] Similar effects probably occur in animals but would be difficult to detect. Additionally, in humans, the bacillus Calmette-Gérin (or BCG) has been associated with uveitis; cyclophosphamide has been associated with dry eye; cisplatin has been associated with neuroretinal toxicity; doxorubicin has been associated with excessive lacrimation and conjunctivitis; 5-fluorouracil has been associated with blurred vision, excessive lacrimation, blepharitis, conjunctivitis, and keratitis; and vincristine has been associated with cranial nerve palsies, optic neuropathy, and cortical and night blindness.[68]

COMPARATIVE ASPECTS

Malignant melanoma of the chorioid and ciliary body is the most common primary ocular malignancy in adult humans. Initially the enucleation of these patients was believed to enhance the risk of metastasis, so a large, randomized clinical trial (the Collaborative Ocular Melanoma Study) comparing enucleation to Iodine-125 brachytherapy, which left the globe intact, was conducted.[69-70] Both treatment modalities were found to yield similar results, although many patients still died from metastatic melanoma and it appears that significant improvement in survival rates will depend on developing effective systemic therapeutic modalities for melanoma.[69-70] Retinoblastoma is the most common malignant intraocular tumor of children, and it has a genetic basis. One case of retinoblastoma has also been described in a dog.[71] With therapy, long-term survival in children is over 85%, but many patients develop second tumors, especially osteosarcoma.[72] Cancer-associated retinopathy is an uncommon, immune-mediated paraneoplastic phenomenon in humans in which antibodies are directed against specific retinal autoantigens such as recoverin.[73-75] In this condition, patients with small-cell lung carcinoma and other tumors may develop blurred vision, impaired color vision, substantial visual field defects, or complete blindness as tumor antigens cross-react with specific retinal components.[73-75] Treatment with intravenous immunoglobulin has been reported to return vision to some patients.[73]

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Hematopoietic Tumors

SECTION A
Canine Lymphoma and Lymphoid Leukemia

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LYMPHOMA

The lymphomas (malignant lymphoma or lymphosarcoma) are a diverse group of neoplasms that have in common their origin from lymphoreticular cells. They usually arise in lymphoid tissues, such as lymph nodes, spleen, and bone marrow; however, they may arise in almost any tissue in the body. Lymphoma is one of the most common neoplasms in the dog. The annual incidence has been estimated at 13 to 24 cases per 100,000 dogs at risk.[1,2] The annual incidence rates at specific ages are estimated to be 1.5 per 100,000 for dogs less than 1 year old and 84 per 100,000 in the 10- to 11-year-old age group.[3] Lymphoma accounts for approximately 7% to 24% of all canine neoplasia and 83% of all canine hematopoietic malignancies.[4,5] In a review of the Veterinary Medical Data Base (VMDP) program, based at Purdue University, from 1987 to 1997, the frequency with which canine lymphoma patients were presented to 20 veterinary institutions increased from 0.75% of the total caseload to 2%, and the frequency apparently continues to increase. A similar trend is present in physician-based oncology. Non-Hodgkin's lymphoma accounts for 5% of all new cancer cases; it is the fifth leading cause of cancer death and the second fastest growing cancer in terms of mortality in humans.[6]

Lymphoma affects primarily middle-aged to older dogs (median age, 6 to 9 years).[1,7] A lower risk has been reported for intact females,[8] but most reports show that gender is not an important risk factor.[7,8b] Dog breeds reported to have a higher incidence of lymphoma include boxers, bull mastiffs, basset hounds, Saint Bernards, Scottish terriers, Airedales, and bulldogs; breeds with a lower risk include dachshunds and Pomeranians.[8,9]

Etiology

The etiology of canine lymphoma is largely unknown and likely multifactorial; however, current investigations are shedding significant light on the subject.

Genetic and molecular factors

Recent advances in molecular cytogenetics (see Chapter 1, section A), including gene microarray techniques, have been and currently are being applied to investigations of chromosomal aberrations in dogs with lymphoma.[10-12] Breen's group has documented gain of canine chromosomes 13 and 31 and loss of chromosome 14 as the most common aberrations in a group of 25 cases analyzed.[11] The recent publication of the canine genome and the commercial availability of canine gene microarrays (e.g., GeneChip Canine Genome 2.0 array; Affymetrix Santa Clara, California) certainly will lead to advances in our understanding of the genetic events in lymphoma in the very near future.[13] Several genetic predispositions have been reported for a pedigree of bull mastiffs,[14] a group of related otter hounds, a family of rottweilers, and a breeding pair of unrelated Scottish terriers.[15] Germ line and somatic genetic mutation in the p53 tumor suppressor gene (see Chapter 2) and the N-*ras* gene have been documented in bull mastiffs and in a dog with lymphoma, respectively.[16-18]

In addition, differences in the prevalence of immunophenotypic subtypes of lymphoma among different breeds have been shown to indicate heritable risks.[19,19b] Epigenetic modifications also have been investigated in dogs with lymphoma; deoxyribonucleic acid (DNA) hypomethylation (see Chapter 1, section A) was a feature of neoplastic cells in most lymphoma cases and in one third of the leukemia cases investigated and likely is involved in malignant transformation of lymphoid cells.[20]

In humans, characteristic chromosomal abnormalities are being described with increasing frequency as more precise banding and other high-resolution techniques are applied. Chromosomal aberrations are nonrandom in human lymphoma, and several aberrations serve as markers for various subtypes of lymphoma. In addition, several oncogenes that may play a role in the pathogenesis of lymphoma have been detected based on the identification of cytogenetic abnormalities.[21-23] Chromosomal aberrations also have been reported in canine lymphoma.[10-12,24] A study of 61 dogs with lymphoma demonstrated a treatment advantage in dogs with trisomy of chromosome 13 (25% of the dogs studied), as evidenced by an increase in duration of the first remission and overall survival time.[24]

As our knowledge of molecular events and tumorigenesis has expanded, several molecular aberrations have been implicated in various canine tumor types, and some associated with lymphoma have been identified. Altered oncogene/tumor suppressor gene expression, epigenetic changes, signal transduction, and death-pathway alterations are common in human lymphomas and likely are also involved in the dog. As mentioned earlier, N-*ras* and p53 aberrations, although rare in dogs, have been implicated in some dogs with lymphoma.[16-18,25-27] Telomerase activity (see Chapter 14, section D) also has been documented in canine lymphoma tissues.[28-30] Alterations in cellular death-pathways, particularly the Bcl-2 family of proapoptotic and antiapoptotic governing molecules, have been implicated in human non-Hodgkin's lymphoma[31] and currently are under investigation in canine lymphoma.

Infectious factors

The hypothesis that a retrovirus may be involved in the pathogenesis of canine lymphoma has not been confirmed.[32] However, viral particles with properties similar to those of retroviruses have been identified in short-term cultures of canine lymphoma tissue.[33-36]

In physician-based oncology, a direct association has been made between *Helicobacter* infections and the development of gastric lymphoma.[37] Although this has not been shown definitively in dogs, evidence indicates that *Helicobacter* infection in laboratory beagles results in gastric lymphoid follicle formation, which is considered a precursor of mucosa-associated lymphoid tissue (MALT) lymphoma in humans.[38]

Environmental factors

Some evidence has accumulated that implicates phenoxyacetic acid herbicides, particularly 2,4-dichlorophenoxyacetic acid (2,4-D), in the development of human non-Hodgkin's lymphoma.[39,40] A population case control study of non-Hodgkin's lymphoma in Kansas farmers reported a twofold to sixfold higher risk in individuals who frequently mixed or applied herbicides (specifically 2,4-D).[41] A published, hospital-based case control study of dogs indicated that owners in households with dogs that developed malignant lymphoma applied 2,4-D herbicides to their lawn or employed commercial lawn care companies to treat their yard more frequently than owners of dogs without lymphoma.[42] The risk of canine lymphoma was reported to rise twofold (odds ratio, 1.3) with four or more yearly applications of 2,4-D. The results of this study since have drawn criticism, and three follow-up investigations have not validated the assertion of increased risk.[43-45]

In another study, dogs exposed to lawn treatment within 7 days of application were more than 50 times more likely to have urine levels of 2,4-D of 50 µg/L or higher.[46] The highest concentration was noted 2 days after application. In a study of 28 dogs with lymphoma, the tumors of 20 dogs with known exposure to 2,4-D were analyzed using polymerase chain reaction (PCR) technology for cellular N-*ras* oncogene mutations.[47] The *ras* genes influence cell proliferation and may induce differentiation through signal transduction pathways. Mutation in the *ras* genes results in *ras* proteins that promote cell growth. One dog in the series showed a mutation of N-*ras*, indicating that such mutations are uncommon in canine lymphoma, a finding similar to that for humans with lymphoma.[47]

In an environmental case control study performed in Europe, two variables, residency in industrial areas and use of chemicals (defined as paints or solvents) by owners, modestly increased the risk of lymphoma; however, no link was found between the use of pesticides and risk.[48]

A weak association between lymphoma in dogs and exposure to strong magnetic fields was observed in a preliminary epidemiologic study.[49] In this hospital-based, case control study, dogs categorized as having high or very high exposure had an increased risk of lymphoma (odds ratio, 1.8). More thorough studies are necessary to evaluate this association further.

Immunosuppression

Impaired immune function has been identified in dogs with lymphoma.[50,51] Immune system alterations in the dog (e.g., immune-mediated thrombocytopenia), independent of age and gender, have been associated with a higher risk of subsequent development of lymphoma compared to the normal population.[52,53] Additional evidence for the role of the immune system in the development of lymphoma comes from observation in human transplantation patients. Individuals with immunosuppression have a higher risk of lymphoreticular cancer,[54,55] and organ transplant patients have a higher incidence of non-Hodgkin's lymphoma.[56] A case of lymphoma that developed in a dog after treatment with cyclosporine, although only one case, supports a link to immunosuppressive therapy in the species.[57]
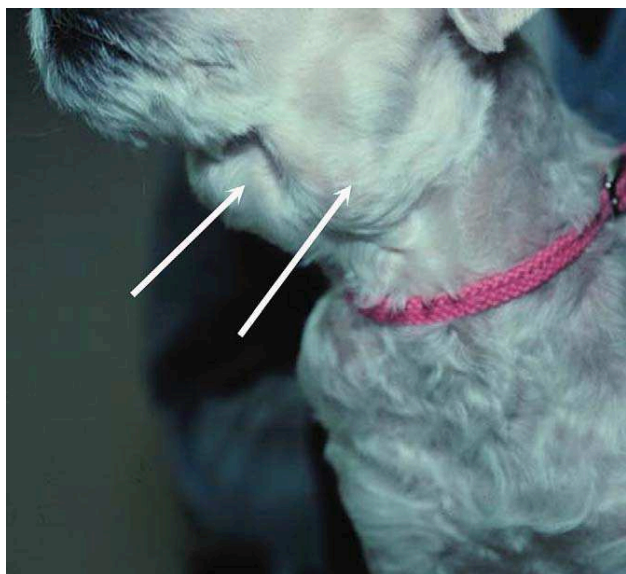
Figure 31-1

A dog with obvious mandibular lymphadenopathy resulting from multicentric lymphoma.

Figure 31-2

Lateral thoracic radiograph of a dog with diffuse interstitial infiltration with lymphoma secondary to multicentric lymphoma.

Pathology and Natural Behavior

The classification of malignant lymphoma in dogs can be distinguished on the basis of anatomic location, histologic criteria, and immunophenotypic characteristics. The most common anatomic forms of lymphoma, in order of decreasing prevalence, are the multicentric, craniomediastinal, gastrointestinal, and cutaneous forms. Primary extranodal forms, which can occur in any location outside the lymphatic system, include the eyes, central nervous system (CNS), bone, testes, bladder, heart, and nasal cavity.

Eighty percent of dogs with lymphoma develop the multicentric form, which is distinguished by the presence of superficial lymphadenopathy (Figure 31-1).[58] Lymph node enlargement usually is painless, rubbery, and discrete and may be localized initially to the mandibular and prescapular nodes. Most animals are asymptomatic at the time of presentation, but approximately 20% to 40% have a history of weight loss, lethargy, anorexia, and febrile episodes.[59,60] Diffuse pulmonary infiltration also may be seen in 27% to 34% of affected dogs, as detected by radiographic changes (Figure 31-2).[61-64] Based on bronchoalveolar lavage, the actual incidence of lung involvement may be higher.[62,65] Hepatosplenomegaly is the most common manifestation of abdominal involvement and usually is associated with an advanced stage of multicentric disease.

The alimentary form of lymphoma is much less common, usually accounting for 5% to 7% of all canine lymphomas.[58] This form is reported to be more common in male dogs than female dogs,[8] similar to observations in humans.[66] Dogs with infiltrative disease of the intestinal tract show weight loss, anorexia, panhypoproteinemia, and evidence of malabsorption.[67,68] Primary gastrointestinal (GI) lymphoma in dogs usually occurs multifocally and diffusely throughout the submucosa and lamina propria of the small intestine, with frequent superficial ulceration and occasional transmural infiltration of the serosa. Lymphocytic-plasmacytic inflammation can be seen adjacent to or distant from the primary tumor.[67] Pathologically, some of these neoplasms may resemble plasma cell tumors, and aberrant production of immunoglobulins may occur.

Histopathologically, distinguishing between gastrointestinal lymphoma and lymphocytic-plasmacytic enteritis (LPE) can be difficult.[67] Some have suggested that LPE may be a prelymphomatous change in the GI tract. A syndrome of immunoproliferative intestinal disease characterized by lymphocytic-plasmacytic enteritis has been described in basenjis, which subsequently develop gastrointestinal lymphoma.[69] In addition, plasma cell–rich areas with heterogeneous lymphomatous infiltration may resemble lesions of LPE.

Only a few reports specifically identify the immunophenotype of the lymphocyte subpopulations in alimentary lymphoma. Historically, it was presumed that they most likely originate from B cells; however, recent evidence suggests that most gastrointestinal lymphomas in dogs originate from T cells.[70] The boxer and Shar-pei breeds appear to be overrepresented in alimentary lymphoma and are reported to have morphologic features (epitheliotropism) consistent with T-cell disease.[71]

A   B
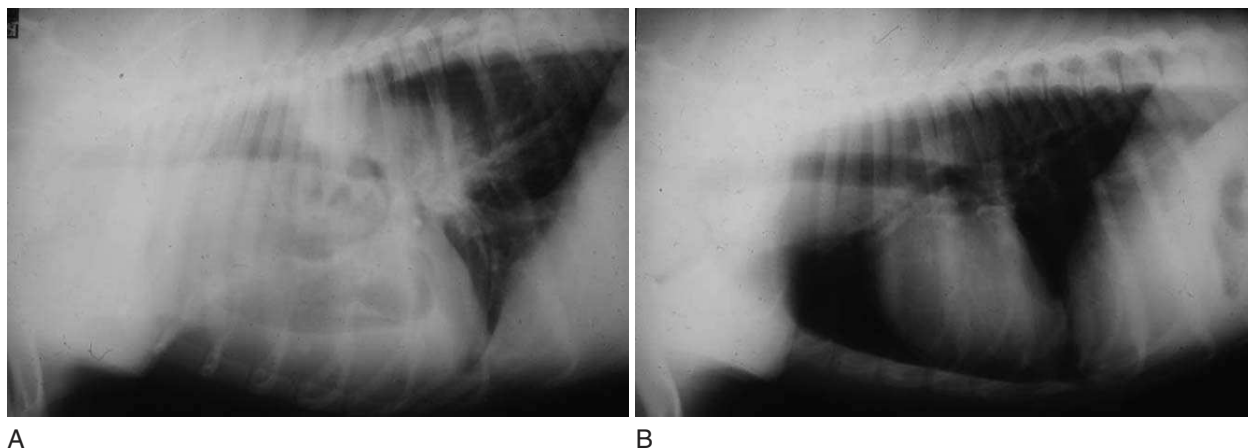
Figure 31-3

A, Lateral radiograph of a dog with mediastinal lymphoma. **B,** Radiographs of the same dog after complete remission was achieved with chemotherapy.

The mediastinal form of lymphoma occurs in approximately 5% of cases.[58] This form is characterized by enlargement of the craniomediastinal lymph nodes or the thymus, or both (Figure 31-3). However, as previously noted, 20% of dogs with multicentric lymphoma have radiographic evidence of craniomediastinal lymphadenopathy.[63] Hypercalcemia is reported to occur in 10% to 40% of dogs with lymphoma and is most common with the mediastinal form.[72,73] In a study of 37 dogs with lymphoma and hypercalcemia, 16 (43%) had mediastinal lymphoma.[74] The mediastinal form in dogs is most commonly associated with a T-cell phenotype.[60,75]

Cutaneous lymphoma can be solitary or more generalized and usually is classified as epitheliotropic (mycosis fungoides) or nonepitheliotropic.[76-84] Cutaneous lymphoma may also involve the oral mucosa,[76] and extracutaneous involvement can occur, most often in the lymph nodes, spleen, liver, and bone marrow. Canine epitheliotropic cutaneous lymphoma is the most common form of cutaneous lymphoma and usually originates from T cells,[85] similar to its development in humans. In dogs the T cells are CD8+ cells, whereas in humans they are mostly CD4+ cells.[86] A rare form of cutaneous T-cell lymphoma is characterized by generalized skin involvement with evidence of circulating malignant T cells in the peripheral blood. These lymphocytes usually are large (15 to 20 μm in diameter) and have folded, grooved nuclei. In humans this is called *Sézary syndrome*,[87] which also has been reported in dogs and cats.[79,80,88,89] B-cell cutaneous lymphomas usually spare the epidermis and papillary dermis and affect the middle and deep portions of the dermis.

Hepatosplenic lymphoma is a relatively uncommon, distinct presentation in the dog marked by a lack of peripheral lymphadenopathy in the face of hepatic, splenic, and bone marrow infiltration with malignant lymphocytes, usually of T-cell origin. Biologically, this form of lymphoma is extremely aggressive and poorly responsive to therapy. In humans the tumor usually is composed of $\gamma\delta$T cells (i.e., T cells that express the $\gamma\delta$T-cell receptor), and this immunophenotype has been confirmed in at least one dog in the veterinary literature.[90-92]

Intravascular (angiotrophic, angioendotheliomatosis) lymphoma is a distinct form of lymphoma defined as proliferations of neoplastic lymphocytes within the lumen and wall of blood vessels in the absence of a primary extravascular mass or leukemia. It has been reported several times in the veterinary literature, and in most cases it involves the central and peripheral nervous system (including the eye).[93-98] The B-cell immunophenotype is most common in humans; however, in most reported cases in dogs, the origin is either T cell or *null cell* (neither B nor T cell), although one case of a B-cell phenotype has been reported.

Histologic Classification Systems

Lymphomas arise from a clonal expansion of lymphoid cells with distinctive morphologic and immunophenotypic features. Many histologic systems have been used to classify non-Hodgkin's lymphoma (NHL) in humans, and some of these have been applied to lymphoma in the dog and other species.[99-107] The National Cancer Institute (NCI) of Working Formulation and the updated Kiel system have been adapted to canine tumors with some success (Tables 31-1 and 31-2). The World Health Organization (WHO) also publishes a histologic classification scheme, which uses the revised European American lymphoma (REAL) system as a basis for defining

TABLE 31-1   Classification of Canine Lymphoma by the National Cancer Institute Working Formulation

Grade	Category	PERCENTAGE		
		Greenlee et al. (n = 176)	Carter et al. (n = 285)	Teske et al. (n = 116)
Low grade		11	5.3	16.3
	Small lymphocytic	10	4.9	—
	Follicular small cleaved	—	0	12
	Follicular mixed small cleaved	1	0.4	4.3
Intermediate grade		59.9	28.4	74.6
	Follicular large cell	3.4	0.4	31
	Diffuse small cleaved cell	3.4	5.9	8.6
	Diffuse mixed small and large	5.1	2.1	5
	Diffuse large cell	48	20	30
High grade		29.4	66.3	6
	Diffuse immunoblastic	25.6	24.9	6
	Diffuse lymphoblastic	0.6	17.2	—
	Diffuse small noncleaved	3.2	24.2	—

Modified from Greenlee PG, Filippa DA, Quimby FW et al: Lymphoma in dogs: a morphologic, immunologic, and clinical study, Cancer 66: 480-490, 1990; Carter RF, Valli VE, Lumsden JH: The cytology, histology and prevalence of cell types in canine lymphoma classified according to the National Cancer Institute Working Formulation, Can J Vet Res 50:154-164, 1986; and Teske E, van Heerde P, Rutteman GR et al: Prognostic factors for treatment of malignant lymphoma in dogs, J Am Vet Med Assoc 205:1722-1728, 1994.

TABLE 31-2   Kiel System for Classifying Canine Lymphomas

Category	PERCENTAGE	
	Greenlee et al. (n = 176)	Teske et al. (n = 95)
Low grade	24.4	29.5
Lymphocytic	6.8	—
Lymphoplasmacytic	3.4	7.3
Centrocytic	4	19
Centroblastic-centrocytic	10.2	3.2
High grade	47.2	70.5
Centroblastic	47.2	65.2
Lymphoblastic T cell	0.6	—
Lymphoblastic B cell	2.2	—
Immunoblastic	25.6	5.3

Modified from Greenlee PG, Filippa DA, Quimby FW et al: Lymphoma in dogs: a morphologic, immunologic, and clinical study, Cancer 66:480-490, 1990; and Teske E, van Heerde P, Rutteman GR et al: Prognostic factors for treatment of malignant lymphoma in dogs, J Am Vet Med Assoc 205: 1722-1728, 1994.

histologic categories of hematopoietic tumors in domestic animals.[107] This system incorporates both histologic and immunohistologic criteria (B- and T-cell immunophenotype). The clinical relevance of this system is likely to be high; however, it awaits further investigation. More recently, the Oncology Committee of the American College of Veterinary Pathologists (ACVP) has established a Lymphoma Subcommittee to formulate a classification system for lymphoma that has clinical relevance. The subcommittee has obtained information from an international group of veterinary oncologists and pathologists, and its report is expected to be completed by 2008.

The Working Formulation (WF) was developed to allow investigators to "translate" among the numerous classification systems so that clinical trials could be compared in humans. Most of the larger compilations agree that most canine lymphomas are intermediate or high grade; however, diffuse immunoblastic forms appear to predominate in the United States, whereas the follicular large cell variations predominate in Europe. A comparison of European and American classifications is warranted based on this discrepancy. The WF categorizes tumors according to pattern (diffuse or follicular) and cell type (e.g., small cleaved cell, large cell, immunoblastic), but it does not include information

about the immunophenotype of the tumor.[103] The WF subtypes are related to the biology of the tumor and patient survival. The updated Kiel classification includes the architectural pattern, morphology (centroblastic, centrocytic, or immunoblastic), and immunophenotype (B cell or T cell) of the tumor cells.[102] In both systems, the tumors then can be categorized as low-grade, intermediate-grade, or high-grade malignancies. Low-grade lymphomas composed of small cells with a low mitotic rate typically progress slowly and are associated with long survival times but are incurable. High-grade lymphomas with a high mitotic rate progress rapidly but are more likely to respond to chemotherapy and, in humans, are potentially curable.

Several features of canine lymphomas become apparent when the WF or updated Kiel classification is applied. The most striking difference between canine and human lymphomas is the scarcity of follicular lymphomas in the dog.[108-111] Some diffuse lymphomas in the dog initially may be follicular, but these may progress to the more aggressive, diffuse form by the time of diagnostic biopsy. Only a small percentage of canine lymphomas (5.3% to 29%) are considered low-grade tumors.[60,104,105,112] Most low-grade small cell lymphomas are T cell in origin.[105]

High-grade lymphomas occur frequently if the diffuse large cell lymphomas, classified as intermediate-grade in the WF, are considered high-grade, as in the updated Kiel classification (in which they are labeled *diffuse centroblastic lymphomas*). Canine lymphoblastic lymphomas are uncommon.[72,104] Most high-grade lymphomas are of B-cell origin.[105] However, a documented difference exists in the prevalence of the various lymphoma immunophenotypes based on breed.[19] For example, cocker spaniels and Doberman pinschers are more likely to develop B-cell lymphoma, boxers are more likely to have T-cell lymphoma, and golden retrievers appear to have an equal likelihood of B- and T-cell tumors.

To be clinically useful, these classification systems in the end must yield information about response to therapy, maintenance of remission, and survival. Some studies suggest that the subtypes in the WF can be correlated with survival, and the Kiel system may be useful for predicting relapse.[113,114] In most studies, high-grade lymphomas show a complete response to chemotherapy significantly more often than low-grade tumors. However, dogs with low-grade tumors may live a long time without aggressive chemotherapy. Dogs with T-cell lymphomas have shown a lower rate of complete response to chemotherapy and shorter remission and survival times than dogs with B-cell tumors.[60,75,112,113] Furthermore, T-cell lymphomas tend to be associated with hypercalcemia.[115-117]

In the veterinary literature, 60% to 80% of lymphoma cases in dogs are B-cell lymphoma; T-cell lymphomas account for 10% to 38%; mixed B- and T-cell disease accounts for as many as 22%; and null cell tumors (i.e., neither B-cell nor T-cell immunoreactive) represent fewer than 5%.* The development of monoclonal antibodies to detect specific markers on canine lymphocytes has made immunophenotyping of tumors in dogs routinely available in many commercial laboratories. Such techniques also can be performed on paraffin-embedded samples and on cytologic specimens obtained by fine-needle aspiration.[118-122]

The Rappaport classification system, proposed in 1956 for human NHL, described the architectural pattern (follicular or diffuse) and the cytologic features (well differentiated, poorly differentiated, or histiocytic) of the tumors.[99,123] The term *histiocytic* was applied to tumors in which most of the lymphocytes had larger diameters, more vesicular nuclei, and more prominent nucleoli than those of lymphocytic or undifferentiated lymphomas; it also was used because the malignant cells have some morphologic features of benign histiocytes. However, immunophenotyping has failed to document a biologic relationship between these cells and true histiocytes, therefore the term now is largely considered a misnomer. Furthermore, this subgroup of histiocytic lymphomas included tumors with different morphologic and immunophenotypic features. The Rappaport classification has not been useful in providing prognostic information or in guiding therapy in dogs with lymphoma because of the low number of follicular tumors in dogs, the problematic "histiocytic" subgroup, and the failure to account for different morphologic and immunologic cell types.[100]

One criticism of these classification systems is that they fail to include extranodal lymphomas as a separate category. Although differences between nodal and extranodal tumors in biologic behavior and prognosis are well recognized, comparative information about the histogenesis of these tumors has been lacking. For example, in humans, small cell lymphomas arising from MALT are composed of cells with a different immunophenotype from that of other small cell lymphomas (i.e., MALT lymphomas typically are negative for both CD5 and CD10).[124-126] Except for cutaneous lymphoid neoplasms, detailed characterization of extranodal lymphomas in dogs has not been done. Although cutaneous lymphoma is a heterogeneous group of neoplasms that includes an epitheliotropic form resembling mycosis fungoides and a nonepitheliotropic form, most cutaneous lymphomas have a T-cell phenotype.[85,127]

To summarize, it is important to determine the histologic grade of canine lymphomas as low (small lymphocytic or centrocytic lymphomas) or intermediate

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*References 60, 72, 75, 105, 112, 113, and 118-120.

to high (diffuse large cell, centroblastic, and immunoblastic lymphomas). Furthermore, determining the immunophenotype of the tumor provides useful information. Response rates to chemotherapy are better in animals with B-cell tumors and intermediate- to high-grade lymphomas. Dogs with low-grade lymphomas can have long survival times without aggressive therapy.

History and Clinical Signs

The clinical signs associated with canine lymphoma are variable and depend on the extent and location of the tumor. Multicentric lymphoma is the most common form (80%), and generalized painless lymphadenopathy (see Figure 31-1) is the most consistent finding. In addition, hepatosplenomegaly and bone marrow involvement are common. Most dogs with multicentric lymphoma do not have dramatic signs of systemic illness (WHO substage *a*) (Box 31-1), however, a large array of nonspecific signs can occur, such as anorexia, weight loss, vomiting, diarrhea, emaciation, ascites, dyspnea, polydipsia, polyuria, and fever (WHO substage *b*).[59,60,128-130] Dogs with T-cell lymphoma are more likely to have constitutional signs (i.e., substage *b*).[118] Polydipsia and polyuria are particularly evident in dogs with hypercalcemia of malignancy. Dogs also may have a history of or clinical signs related to blood dyscrasias secondary to marked tumor infiltration of the bone marrow (myelophthisis) or paraneoplastic anemia, thrombocytopenia, or neutropenia. These signs could include fever, sepsis, anemia, and hemorrhage. Dogs with gastrointestinal or alimentary lymphoma usually have nonspecific GI signs, such as vomiting, diarrhea, weight loss, and malabsorption.[66-68] The mesenteric lymph nodes, spleen, and liver may be involved.

The mediastinal form of lymphoma is characterized by enlargement of the craniomediastinal structures or thymus or both (see Figure 31-3, *A*), and clinical signs are associated with the extent of disease or polydipsia and polyuria from hypercalcemia. These patients commonly have respiratory distress caused by a space-occupying mass and pleural effusion, exercise intolerance, and possibly regurgitation. Dogs with mediastinal lymphoma also may have precaval syndrome, characterized by pitting edema of the head, neck, and forelimbs secondary to tumor compression or invasion of the cranial vena cava (Figure 31-4).

Signs in dogs with extranodal lymphoma depend on the specific organ involved. Cutaneous lymphoma usually is generalized or multifocal.[76-84] Tumors occur as nodules, plaques, ulcers, and erythremic or exfoliative dermatitis. Epitheliotropic T-cell lymphoma (e.g., mycosis fungoides) has a chronic clinical course with three apparent clinical stages. Initially, scaling, alopecia, and pruritus are seen (Figure 31-5, *A*). As the disease progresses, the skin becomes more erythematous, thickened, ulcerated, and exudative. The final stage is characterized by proliferative plaques and nodules with progressive ulceration (Figure 31-5, *B*). Oral involvement also may occur, which can appear as multicentric, erythematous, plaquelike lesions or nodules on the gums and lips (Figure 31-5, *C*).[76]

Dogs with primary CNS lymphoma may have either multifocal or solitary involvement.[131-133] Seizures, paralysis, and paresis may be noted. Ocular lymphoma is characterized by infiltration and thickening of the iris,

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Box 31-1	**World Health Organization Clinical Staging System for Lymphosarcoma in Domestic Animals**

Anatomic site
A. Generalized
B. Alimentary
C. Thymic
D. Skin
E. Leukemia (true)*
F. Others (including solitary renal tumors)

Stage (includes anatomic site)
I   Involvement limited to a single node or lymphoid tissue in a single organ†
Ia   Stage I without systemic signs
Ib   Stage I with systemic signs
II   Involvement of many lymph nodes in a regional area (with or without the tonsils)
IIa   Stage II without systemic signs

IIb   Stage II with systemic signs
III   Generalized lymph node involvement
IIIa   Stage III without systemic signs
IIIb   Stage III with systemic signs
IV   Liver and/or spleen involvement (with or without stage III disease)
IVa   Stage IV without systemic signs
IVb   Stage IV with systemic signs
V   Manifestation in the blood and involvement of bone marrow and/or other organ systems (with or without stages I to IV disease)
Va   Stage V without systemic signs
Vb   Stage V with systemic signs

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*Only blood and bone marrow involved.
†Excluding bone marrow.

A,

B

Figure 31-4

A, Facial edema in a dog with precaval syndrome secondary to mediastinal lymphoma. **B,** Forelimb edema in a dog with precaval syndrome secondary to mediastinal lymphoma. **C,** The dog in **B** 24 hours after radiation therapy to the craniomediastinal mass, showing resolution of pitting edema.

C

uveitis, hypopyon, hyphema, posterior synechia, and glaucoma (Figure 31-6).[134] In one study of 94 cases of canine multicentric lymphoma, 37% had ocular changes consistent with lymphoma, and in a series of 102 cases of uveitis in dogs, the condition occurred secondary to lymphoma in 17% of the cases.[135,136] Anterior uveitis was most often seen in advanced stage disease (stage V). Dogs with intravascular lymphoma usually have signs related to CNS, peripheral nervous system (PNS), or ocular involvement,[93-98] including paraparesis, ataxia, hyperesthesia, seizures, blindness, lethargy, anorexia, weight loss, diarrhea, polyuria, polydipsia, and intermittent fever. Dogs with pure hepatosplenic lymphoma usually have nonspecific signs such as lethargy, inappetence, and weakness.

The differential diagnosis of lymphadenopathy depends on the dog's travel history (i.e., relative to infectious disease) and the size, consistency, and location of the affected lymph nodes. Other causes of lymphadenopathy include bacterial and viral infections, parasites (*Toxoplasma* and *Leishmania* spp.), rickettsial organisms (salmon poisoning, *Ehrlichia* sp.), and fungal agents (*Blastomyces* and *Histoplasma* spp.). The potential for hypercalcemia to accompany systemic
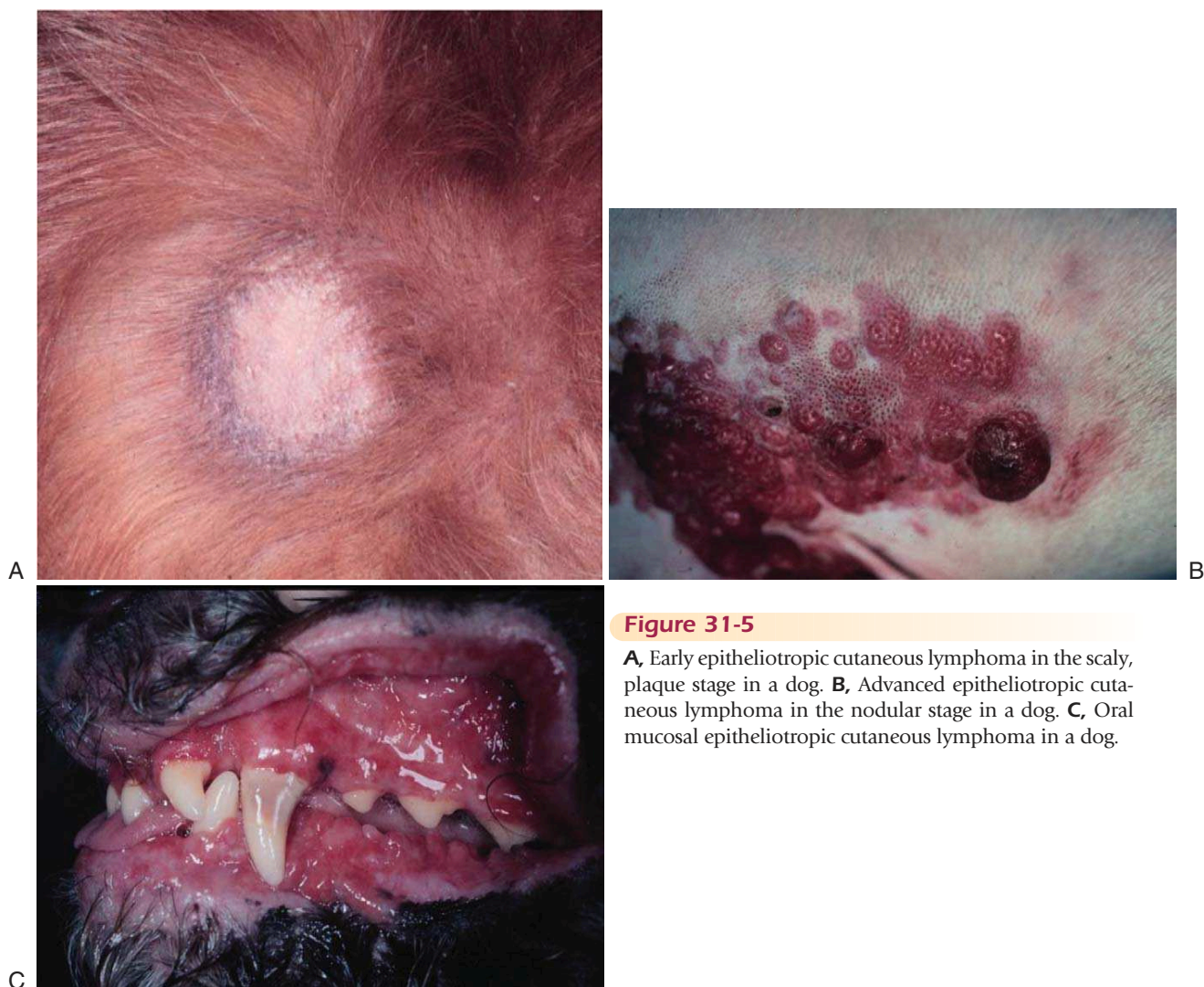
Figure 31-5

A, Early epitheliotropic cutaneous lymphoma in the scaly, plaque stage in a dog. **B,** Advanced epitheliotropic cutaneous lymphoma in the nodular stage in a dog. **C,** Oral mucosal epitheliotropic cutaneous lymphoma in a dog.

Figure 31-6

Ocular lymphoma involving the anterior chamber.

fungal diseases may further complicate differentiation from lymphoma. Discrete, hard, asymmetric lymph nodes, particularly if fixed to underlying tissues, may indicate metastatic tumors, such as mast cell tumors or carcinomas. Immune-mediated diseases (e.g., pemphigus, systemic lupus erythematosus) also may cause mild to moderately enlarged lymph nodes. The various diseases or conditions that can be misdiagnosed as canine lymphoma are listed in Box 31-2.

Canine lymphoma also may be associated with paraneoplastic syndromes (see Chapter 5). Anemia is the most common lymphoma-related paraneoplastic syndrome.[137,138] Paraneoplastic hypercalcemia is also common in dogs with lymphoma. Hypercalcemia is characterized clinically by anorexia, weight loss, muscle weakness, lethargy, polyuria, polydipsia and, rarely, CNS depression and coma.[117,139,140] In most cases lymphoma-induced hypercalcemia results from a

Box 31-2	Disorders That May be Misdiagnosed as Canine Lymphoma

Form of lymphoma	Possible misdiagnosis
Multicentric lymphoma	• Disseminated infections (e.g., bacterial, viral, rickettsial, parasitic, fungal) • Immune-mediated disorders (e.g., dermatopathies, vasculitis, polyarthritis, lupus) • Tumors metastatic to nodes • Other hematopoietic tumors (e.g., leukemia, multiple myeloma, malignant or systemic histiocytosis)
Mediastinal lymphoma	• Other tumors (e.g., thymoma, chemodectoma, ultimobranchial cyst, ectopic thyroid carcinoma, pleural carcinomatosis, pulmonary lymphomatoid granulomatosis*) • Infectious disease (e.g., granulomatous disease, pyothorax) • Miscellaneous (e.g., congestive heart failure, chylothorax, hemothorax)
Alimentary lymphoma	• Other gastrointestinal tumors, foreign body, lymphangiectasia, lymphocytic-plasmacytic enteritis, systemic mycosis, gastroduodenal ulceration
Cutaneous lymphoma	• Infectious dermatitis (e.g., advanced pyoderma) • Immune-mediated dermatitis (e.g., pemphigus) • Other cutaneous neoplasms
Extranodal lymphoma	• Varies, depending on the organ or system involved

Researchers debate whether this disease actually exists; in most cases it has been reclassified as a lymphoid neoplasia.

hormone-like substance, parathyroid hormone–related peptide (PTHrP), elaborated by neoplastic cells; however, it also can be related to the production of several other humoral factors, including interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and vitamin D analogs (e.g., 1,25-dihydroxyvitamin D).[125,141-144] Several investigators have reported that hypercalcemia in dogs with lymphoma is most commonly associated with T-cell lymphoma.[60,113,117,118] Other paraneoplastic syndromes that may be encountered include monoclonal gammopathies, neuropathies, and cancer cachexia.[145,146]

Diagnostics and Clinical Staging

For most animals suspected of having lymphoma, the diagnostic evaluation should include a thorough physical examination; complete blood count (CBC) with a differential cell count, including a platelet count; serum biochemistry profile; and urinalysis. Ultimately, obtaining tissue or cytologic specimens for a definitive diagnosis is essential.

Physical examination

A thorough physical examination should include palpation of all assessable lymph nodes, including those palpable by rectal examination; in the authors' experience, a significant proportion of dogs have rectal polyps consisting of aggregates of neoplastic lymphocytes. The mucous membranes should be inspected for pallor, icterus, petechiae, and ulceration, because these signs may indicate anemia or thrombocytopenia secondary to

myelophthisis or immune-mediated disease or may be evidence of major organ failure or uremia. Abdominal palpation may reveal organomegaly, intestinal wall thickening, or mesenteric lymphadenopathy. Thoracic auscultation may reveal the presence of a mediastinal mass or pleural effusion or both. An ocular examination that includes funduscopic assessment may reveal abnormalities such as uveitis, retinal hemorrhage, and ocular infiltration in approximately one third to one half of dogs with lymphoma.[135,136]

Complete blood count, biochemistry profile, and urinalysis

Anemia, the most common lymphoma-related hematologic abnormality,[137,138] usually is normochromic and normocytic (nonregenerative), consistent with anemia of chronic disease. However, hemolytic anemia may also occur, and regenerative anemias may reflect concomitant blood loss or hemolysis. In addition, if significant myelophthisis is present, the anemia may be accompanied by thrombocytopenia and leukopenia. In animals with anemia or evidence of bleeding, a reticulocyte count, platelet count, and coagulation studies also may be indicated.

Thrombocytopenia may be seen in 30% to 50% of cases, but bleeding is seldom a clinical problem.[138,147,148] Neutrophilia can be seen in 25% to 40% of dogs with lymphoma.[138,147] Lymphocytosis is uncommon and occurs in approximately 20% of affected dogs.[138] Circulating atypical lymphocytes may indicate bone marrow involvement and leukemia. Differentiating multicentric lymphoma with bone marrow involvement

(i.e., stage V disease) from primary lymphoblastic leukemia (discussed later) is important, because the prognoses are entirely different. Hypoproteinemia is observed more often in animals with alimentary lymphoma. If the dogs has a high total protein or evidence of an increased globulin fraction on a chemistry profile, serum proteins should be evaluated by serum electrophoresis. Monoclonal gammopathies have been reported to occur in approximately 6% of dogs with lymphoma.[145]

Serum biochemical abnormalities often reflect the anatomic site involved. In addition, approximately 15% of dogs with lymphoma are hypercalcemic (30% to 40% of those with mediastinal involvement and approximately 35% of those with T-cell lymphomas).[74,118,147] In cases of hypercalcemia of unknown origin, lymphoma should always be considered high on the differential disease list, and diagnostics directed at this possibility should be undertaken (see Chapter 5). The presence of hypercalcemia also can serve as a marker for response to therapy. Elevations in serum urea nitrogen and creatinine can occur secondary to renal infiltration with tumor, hypercalcemic nephrosis, or prerenal azotemia from dehydration. Increases in liver-specific enzyme activity or bilirubin concentrations may result from hepatic parenchymal infiltration. Serum globulin elevations, usually monoclonal, occur infrequently with B-cell lymphoma.

A urinalysis is part of the minimum database used to assess renal function and the urinary tract. For example, isosthenuria and proteinuria in the absence of an active sediment may indicate renal disease, and hematuria may result from a hemostatic abnormality. It is important to remember that isosthenuria in azotemic dogs with hypercalcemia does not necessarily indicate renal disease, because the high calcium levels interfere with tubular concentration capabilities through disruption of antidiuretic hormone (ADH) control.

Abnormalities in serum levels of alpha fetoprotein, alpha-1 glycoprotein, zinc, chromium, iron, and endostatin also have been investigated in dogs with lymphoma.[149-152] The clinical and biologic significance of these alterations has yet to be elucidated.

Histologic and cytologic evaluation of lymph nodes

Morphologic examination of the tissue and cells that comprise the tumor is essential to the diagnosis of lymphoma. Care should be taken to avoid lymph nodes from reactive areas (e.g., mandibular lymph nodes); the prescapular or popliteal lymph nodes are preferable. Also, lymphoid cells are fragile, and in preparing smears of aspirated material, only gentle pressure should be applied in spreading the material on the slides. In most cases, a diagnosis of lymphoma can be made on evaluation of fine-needle aspirates of affected lymph nodes or other tissues. Typically, most of the cells are large lymphoid cells

(larger than neutrophils), and they may have visible nucleoli and basophilic cytoplasm (Figure 31-7, *A*) or fine chromatin with indistinct nucleoli. Because tissue architecture is not maintained in cytologic specimens, effacement of the lymph node or capsular disruption cannot be detected. Therefore, marked reactive hyperplasia characterized by increased numbers of large lymphoid cells may be difficult to distinguish from lymphoma, and small cell lymphomas may have few cytologic clues that point to their malignancy. Also, classification of lymphoma into the subcategories that comprise the low-, intermediate-, and high-grade forms, which has been attempted using the cytologic appearance and immunophenotypic analysis,[153] is performed most accurately on histologic sections.

For accurate histopathologic evaluation, an entire lymph node, including the capsule, should be removed, placed in buffered formalin, and submitted to a pathologist. Although needle core biopsies may be satisfactory, it is important to avoid crush artifact or inadequate sample size. Most pathologists prefer whole node biopsies because they provide the maximum amount of information. Effacement of the normal nodal architecture by neoplastic lymphocytes and capsular disruption are characteristic findings (Figure 31-7, *C* and *D*). Diagnostic ultrasonography and ultrasound-guided fine-needle aspiration or needle biopsy have been very useful for evaluation of involvement of the liver, spleen, or abdominal lymph nodes.[154-158] If possible, the diagnosis should be made by sampling peripheral nodes, avoiding percutaneous biopsies of the liver and spleen. However, if there is no peripheral node involvement, it is appropriate to biopsy affected tissues in the abdominal cavity.

Histologic and cytologic evaluation of extranodal sites

With alimentary lymphoma, an open surgical wedge biopsy of the intestine must be obtained, ideally without entering the intestinal lumen, and adequate tissue must be obtained; this is important because of the difficulty involved in differentiating lymphoma from LPE. Endoscopic biopsies may be inadequate because only a superficial specimen is obtained; however, more aggressive endoscopic biopsy techniques combined with more accurate histopathologic assessments are improving the diagnostic yield of these less invasive techniques.[159] In many dogs with primary gastrointestinal lymphoma, an inflammatory, nonneoplastic infiltrate (e.g., LPE) may be misdiagnosed on biopsy specimens that are too superficial.

Cytologic examination of cerebrospinal fluid (CSF), thoracic fluid, or aspirates of an intracavitary mass is indicated in animals with CNS disease, pleural effusion, or an intrathoracic mass, respectively. In one study of dogs with CNS involvement, CSF analysis was diagnostic in seven of eight dogs.[131] The characteristics of the CSF included an elevated nucleated cell count in the
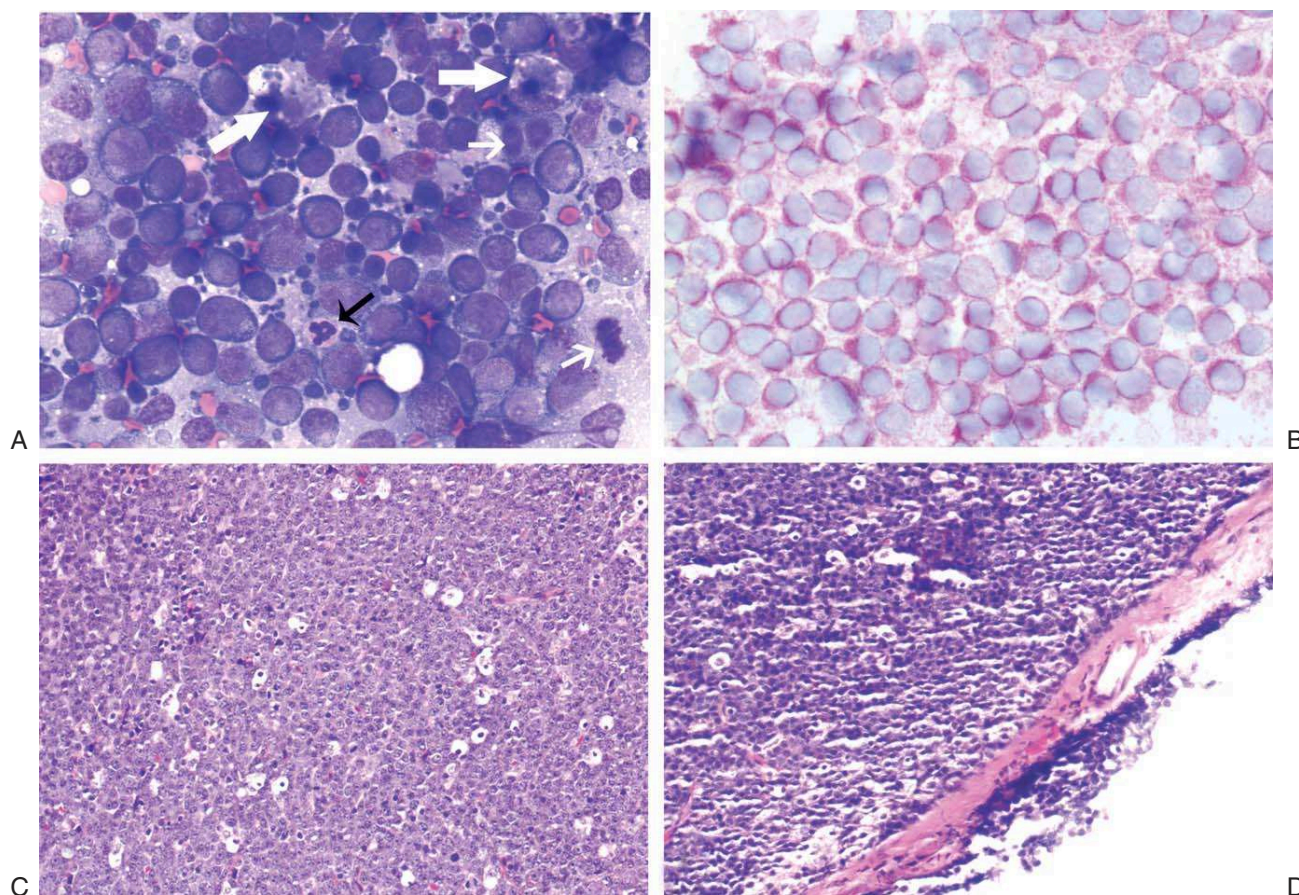
Figure 31-7

Lymph nodes from dogs with lymphoma. **A,** Fine-needle aspirate. Note the homogenous population of large lymphoid cells with prominent nucleoli and basophilic cytoplasm. These cells are larger than the neutrophil *(arrow)* in the field. Mitotic figures *(thin white arrows)* and tingible body macrophages *(thick white arrows)* also are present. (Wright's stain, ×60 objective.) **B,** Fine-needle aspirate stained for CD79a; note that nearly all the lymphocytes are positive. The diagnosis was B-cell lymphoma. (Alkaline phosphatase/fast red, ×60 objective.) **C,** Histologic section; note the effacement of the normal architecture. The white spaces, which are macrophages, give the tissue a "starry sky" appearance. (Hematoxylin and eosin [H&E], ×20 objective.) **D,** Histologic section; note the presence of tumor cells outside the capsule of the lymph node. H&E, ×20 objective.)

seven dogs, and 95% to 100% of the cells were atypical lymphoid cells. The CSF protein concentration was higher in five of seven dogs, ranging from 34 to 310 mg/dl (the reference interval was less than 20 mg/dl).

For cutaneous lymphoma, punch biopsies (3 to 4 mm) should be taken from the most representative and infiltrative, but not infected, skin lesions. Staging procedures for cutaneous lymphoma vary, and the stage has shown no prognostic importance.[160]

Molecular diagnostic techniques

Molecular techniques can be used to establish a diagnosis of lymphoma or to further characterize a tumor after the initial diagnosis has been made. Tissues and cells from peripheral blood, lymph nodes, or other sites can be analyzed by histochemical and cytochemical, immunohistochemical and immunocytochemical, flow cytometric, and PCR techniques. For example, the tumor's immunophenotype (B cell or T cell), proliferation rate (e.g., expression of Ki-67, proliferating cell nuclear antigen [PCNA], and argyrophilic nucleolar organizer regions [AgNORs]), and clonality (PCR for antigen receptor gene rearrangement [PARR]) can be determined.[60,105,113,119-122,161-170] The availability of such analyses is increasing, although currently only the immunophenotype consistently predicts the prognosis in dogs.

Immunophenotyping

Immunophenotyping typically is used to determine the type of cells that make up the lymphoma, but sometimes the technique is helpful for making the diagnosis of lymphoma. When a diverse population of lymphocytes is expected in a tissue, the presence of a homogeneous population of the same immunophenotype is supportive of a neoplastic process. The immunophenotype of a lymphocyte is identified by determining the expression of molecules specific for B cells (e.g., CD79a) and T cells (e.g., CD3). Although tumor cells sometimes have morphologic characteristics that typify a particular immunophenotype, exceptions occur, and morphology cannot be used as the sole determinant of the type of lymphocyte. For example, in a series of nine high-grade T-cell lymphomas and leukemias in dogs, the cells had a plasmacytoid appearance, typically associated with B-cell lymphoma.[171] Similarly, anatomic location does not always predict the immunophenotype. In a series of 44 cases of gastrointestinal lymphoma in dogs, often considered a neoplasm of B cells, the neoplastic cells were identified as T cells (CD3 positivity) in 75% of the cases.[70]

For accurate determination of the immunophenotype, antibodies against lymphocyte markers are applied to tissue sections (immunohistochemistry), cytologic specimens (immunocytochemistry), or individual cells in a fluid medium (flow cytometry). Flow cytometric evaluation of cells from needle aspirates also is feasible.[172] For T cells, markers include CD3 (pan T), CD4 (helper T), and CD8 (cytotoxic T); for B cells, the markers are CD79a (see Figure 31-7, *B*) and CD21.[173] Interestingly, aberrant expression of CD molecules has been reported in canine lymphoma. In a study of 59 dogs with lymphoma, tumor cells from six dogs were positive for both T- and B-cell CD markers; however, a clonality assay (discussed later) revealed clonality for either the T-cell or the immunoglobulin receptor but not both. This indicates that in some cases of B- and T-cell lymphoma, the malignant cells may coexpress B- and T-cell CD markers.[119] Antibodies against these molecules are used to determine the immunophenotype; however, they also have a potential use as a therapeutic modality if tumor cells could be targeted using these antibodies.

Histologic assessment of markers of multidrug resistance and apoptotic pathways (e.g., P-glycoprotein, p53, and Bcl-2 proteins) currently are being evaluated in dogs with lymphoma. However, their significance requires further evaluation.[16,163,174-177]

Clonality assay

Occasionally the diagnosis of lymphoma and the differentiation of malignant verses benign proliferation of lymphocytes is not possible based on standard histologic
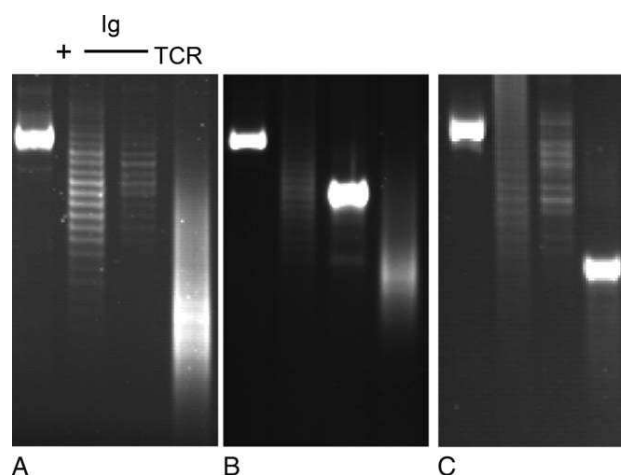
Figure 31-8

Uniform size of polymerase chain reaction (PCR) products as an indicator of clonality. Each panel shows four PCR reactions on a single DNA sample. The first lane (left) in each panel is a positive control that indicates that DNA is present (any nonrearranged gene would be an appropriate target for this reaction). The middle two lanes represent two different reactions that amplify immunoglobulin CDR3, and the fourth lane shows TCRγ CDR3 amplification. The samples are separated on a polyacrylamide gel. **A,** Lymph node aspirate from a normal dog. **B,** Lymph node aspirate from a dog with histologically confirmed multicentric B-cell lymphoma. **C,** Lymph node aspirate from a dog with histologically confirmed T-cell lymphoma. *(From Avery PR, Avery AC: Vet Clin Pathol 33:196-207, 2004.)*

and cytologic criteria. In these cases, advanced molecular analyses are necessary to help confirm a diagnosis. Clonality is the hallmark of malignancy; that is, the malignant cell population theoretically should be derived from expansion of a single malignant clone characterized by a particular DNA region unique to that tumor. For example, in a dog with T-cell lymphoma, all the malignant cells should have the same DNA sequence for the variable region of the T-cell receptor (TCR) gene; likewise, in a dog with B-cell lymphoma, the tumor cells should have identical DNA sequences in the variable region of the immunoglobulin receptor gene. Conversely, in reactive lymphocytosis, the cells are polyclonal for their antigen receptors. Using this knowledge, investigators have used polymerase chain reaction (PCR) PCR techniques to amplify the variable regions of the T-cell and immunoglobulin receptor genes to detect clonal lymphocyte populations in dogs (Figure 31-8).[167-170]

In physician-based medicine, such assays of clonality are approximately 70% to 90% sensitive and have a false positive rate of approximately 5%, and recent studies report similar rates in the dog.[169] False negative and false positive results can occur with clonality assays. For example, cells from a dog with lymphoma may be negative for clonality if the clonal segment of DNA is not detected with the primers used, if the malignant cells are natural killer (NK) cells (rare), or if the malignant cells are present in too low a frequency to be detected.[169] False positive results occur rarely with some infectious diseases, such as ehrlichiosis and Lyme disease. In these cases a diagnosis should be made only after the results of all the diagnostic tests are considered, including histologic and cytologic evaluation, immunophenotyping, and clonality studies, in conjunction with the signalment and physical findings. Molecular techniques, in addition to aiding the diagnosis, could also be useful in determining early recurrence, more accurate clinical staging, and so-called molecular remission rates, because they are more sensitive than standard cytologic assessment for the peripheral blood, bone marrow, and lymph nodes.

Clinical Staging

After a diagnosis has been established, the extent of disease should be determined and correlated to the clinical stage of disease. The WHO staging system routinely used to stage dogs with lymphoma is presented in Box 31-1. Most dogs (more than 80%) are presented in advanced stages (stage III or IV). Some type of imaging and an assessment of bone marrow involvement may be indicated for staging. The degree to which thorough staging is implemented depends on three factors: whether the result will alter the treatment plan; whether relevant prognostic information will be gleamed; and whether the client needs to know. In addition, when different protocols are compared with respect to efficacy, consistent and similar staging systems should be used to avoid so-called stage migration, which results when one staging methodology is more accurate than another.[178] The effect of stage migration currently is being evaluated in veterinary patients with lymphoma, and until the concept has been thoroughly explored, the impact on the prognosis should be considered when different published outcomes are compared.

Bone marrow evaluation

A bone marrow aspirate or biopsy (from the proximal humerus or iliac crest) is indicated for complete staging and in dogs with anemia, lymphocytosis, peripheral lymphocyte atypia, or other peripheral cytopenia. In one study of 53 dogs with lymphoma, 28% had circulating malignant cells and were considered leukemic, whereas bone marrow examination indicated

involvement in 57% of the dogs.[179] The presence of a few prolymphocytes and large lymphocytes with nucleoli in the circulation of dogs with lymphoma may indicate bone marrow involvement. It is important to remember that these cells also can be seen with GI parasitism, immune-mediated hemolytic anemia, and other immune-mediated diseases. Recently, circulating tumor cells in dogs with stage III lymphoma were identified using a clonality assay (PARR).[168] The assay is more sensitive than routine microscopy in detecting malignant cells in circulation, but the correlation of these results with staging and prognosis has not been determined. Bone marrow evaluation offers prognostically valuable information, but if the client is committed to treatment regardless of the stage of disease, it is not necessary.

Imaging

Evaluation of thoracic and abdominal radiographs may be important in determining the extent of internal involvement. Approximately 60% to 75% of dogs with multicentric lymphoma have abnormalities on thoracic radiographs; one third have evidence of pulmonary infiltrates, and two thirds have thoracic lymphadenopathy (sternal and tracheobronchial lymph nodes) and widening of the cranial mediastinum (see Figures 31-2 and 31-3).[61-64] Craniomediastinal lymphadenopathy is detected in 20% of dogs with lymphoma.[63] Abdominal radiographs reveal evidence of sublumbar (iliac) and/or mesenteric lymph node, spleen, or liver involvement in approximately 50% of cases.[63,64] In the authors' practice, in typical cases of canine multicentric lymphoma, imaging is limited to thoracic radiographs because no prognostic difference exists between dogs with stage III disease and those with stage IV disease (i.e., liver or spleen involvement); however, the presence of craniomediastinal lymphadenopathy is prognostically significant (see Prognosis later in this section). If clinical signs attributable to abdominal disease are present, further imaging of the abdomen is warranted. In addition, as stated previously, abdominal ultrasonography can be important for obtaining ultrasound-guided intra-abdominal samples for diagnosis. It is also useful for the diagnosis of gastrointestinal and hepatosplenic lymphoma.[90-92,180,181] Advanced imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), and positron emission/computed tomography (PET/CT), are becoming more commonplace in veterinary practice, and their usefulness is only now being fully determined.[182-186]

Treatment of Multicentric Lymphoma

The therapeutic approach to a particular patient with lymphoma is determined by the stage and substage of disease, the presence or absence of paraneoplastic

disease, the patient's overall physiologic status, the financial and time commitments of the client, and the client's level of comfort with regard to the likelihood of treatment-related side effects. Because most canine lymphomas are intermediate- to high-grade tumors, histopathologic characterization has played a less important role in determining the optimal treatment.

Without treatment, most dogs with lymphoma die of the disease in 4 to 6 weeks.[145] With few exceptions, canine lymphoma is considered a systemic disease and therefore requires systemic therapy to achieve remission and prolonged survival. Systemic chemotherapy continues to be the therapy of choice for canine lymphoma. In general, combination chemotherapy protocols are superior in efficacy to single agent protocols. Single agent protocols, except for doxorubicin, have a lower response rate that is not as durable as combination chemotherapy. In rare cases in which lymphoma is limited to one site (especially an extranodal site), the animal can be treated with a local modality, such as surgery or radiation therapy, as long as the caregiver and clinician are committed to diligent re-evaluation to document subsequent systemic involvement.

Multidrug combination protocols

Many chemotherapy protocols for dogs with lymphoma have been developed over the past 15 to 20 years (Table 31-3).[59,61,72,128,187-201] Most complex combination protocols are modifications of CHOP protocols initially designed for human oncologic use. CHOP represents combinations of cyclophosphamide (C), doxorubicin (represented by the *H*, for hydroxy-daunorubicin), vincristine (O, Oncovin) and prednisone (P). Conventional chemotherapy induces complete remission (CR) in approximately 60% to 90% of dogs, with median survival times of 6 to 12 months depending on the protocol used. Approximately 20% to 25% of dogs live 2 years or longer after initiation of these protocols (Figure 31-9). Response rates and the duration of response vary according to the presence or absence of prognostic factors discussed in the Prognosis section. The cost to the owner depends on the drug or drugs selected, the size of the animal, the frequency of administration, and the laboratory tests needed to monitor toxicity.

Dogs that respond to chemotherapy and achieve complete remission usually are free of clinical signs associated with lymphoma and subsequently return to a very good quality of life. Treating dogs with lymphoma is gratifying, because a high percentage have a complete response. Most dogs tolerate chemotherapy well, and in our experience only a minority of dogs develop significant toxicity. Studies assessing clients' perceptions of medical treatment for cancer in general and lymphoma in particular generally report a positive experience; most owners felt that the treatment was

worthwhile, that it resulted in improvement in their pet's well-being, and that the animal's quality of life during treatment was good.[202,203] Very few clients expressed regret about having the lymphoma treated with a multidrug protocol.

With lymphoma, the fundamental goals of chemotherapy are to induce a complete and durable (longer than 6 months) first remission *(induction)*, to reinduce remission when the tumor recrudesces (or the patient relapses) after achievement of a remission *(reinduction)*, and finally to induce remissions when the cancer fails to respond to induction or reinduction using drugs not present in standard protocols *(rescue)*.

A previously unanswered question in the treatment of lymphoma was whether long-term maintenance chemotherapy was useful after an initial course of aggressive induction chemotherapy that lasted 6 months or less. Long-term maintenance chemotherapy has been shown to be ineffective in humans with Hodgkin's disease, NHL, and multiple myeloma. However, the initial induction course of chemotherapy in humans is much more aggressive than that used in veterinary patients. Although no randomized studies have been performed to address the therapeutic benefit of long-term maintenance chemotherapy in dogs, comparisons of dogs treated with CHOP-based protocols in which all treatment was stopped after 6 months of induction therapy[129] were compared with sequentially treated, historical controls that received a nearly identical protocol that included long-term maintenance therapy.[60] The dogs that received the shortened, less expensive, no-maintenance protocol had comparable remission and survival durations and were more likely to achieve second remissions when they relapsed after completion of chemotherapy than their counterparts that received long-term maintenance therapy. Other studies, although not prospective randomized trials, suggest that aggressive induction or discontinuous therapy (i.e., induction without maintenance) is as good as or superior to protocols that use an extended maintenance phase.[190,193,194,200] These data, taken together, suggest that maintenance therapy is not necessary and indeed may be inappropriate for dogs with lymphoma that are treated with similar combination chemotherapy protocols.

Single agent chemotherapy with known activity for dogs with lymphoma

Currently, the most effective chemotherapeutic agents for lymphoma are doxorubicin, L-asparaginase, polyethylene glycol (PEG)-L-asparaginase,[129,130] vincristine, cyclophosphamide, and prednisone.* Other drugs that also have activity and could be considered second-line

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*References 59, 61, 72, 128, 187-200, 204, and 205.

TABLE 31-3	Summary of Combination or Single Agent Doxorubicin Chemotherapy Protocols for Lymphoma*					
Protocol	Number of Dogs	Remission Rate (%)	Median Remission (mo)	Median Survival (mo)	1-Year Survival (%)	References
COP	20	70	3.3	7.5	<10	188
COP	77	75	6	NR	19	187
A	22	77	6.3	6.3	Not reported (NR)	197
A	21	76	6.8	9	NR	188
A	37	59	4.4	7.7	NR	189
A	121	85	4.3	7.9†	NR	128
A	42	74	4.9	5.7	NR	199
A + piroxicam	33	79	4.3	6.5	NR	199
VMC-L	59	90	4.4	7.3	25	61
VMC-L	147	77	4.7	9.7	25	191
VCA-L	112	73	7.9	11.5	50	72
L-COPA	41	76	11‡	NR	48	192
L-COPA (II)	68	75	9‡	5.8	27 (13 at 2 yr)	193
COPLA/LVP	75	92 (80‡)	5.8	8.4‡	17	198
VELCAP–SC	94	70	5.6	10	44	201
VLCAP–Long	98	69	12.5‡	17‡	NR	194
L-PVCA	24	87	10.3	17	NR	195
L-PVCD	21	71	5	7.6	NR	195
L- VCAM (University of Wisconsin [UW]–Madison)	55	84	8.4	11.9	50 (24 at 2 yr)	59
L-VCA (UW Short)	51	94	9.1	13.2	NR	129

Few of these studies involved a large number of dogs, and fewer compared protocols in a randomized, prospective fashion. Also, staging, inclusion, and response criteria vary considerably among the protocols. For these reasons, evaluation of efficacy is subject to bias, and the various protocols should be compared with caution.

†With COP rescue.

‡These values were given only for cases in which complete remission was reported.

L	L-asparaginase
V	Vincristine
C	Cyclophosphamide
M	Methotrexate
O	Oncovin (vincristine)
P	Prednisone
A	Adriamycin (doxorubicin)
D	Dactinomycin

agents include vinblastine, actinomycin-D, mitoxantrone, chlorambucil, methotrexate, DTIC, 9-aminocamptothecin, ifosfamide, cytosine arabinoside, gemcitabine, lomustine, and dolastatin-10.[206-218] Of these, cytosine arabinoside, ifosfamide, dolastatin-10, and gemcitabine appear to have only minimal activity. Except for doxorubicin, induction with single agent chemotherapy does not result in durable remission durations compared with standard combination protocols. The efficacy of incorporating these newer drugs with single agent activity into standard combination protocols awaits further investigation.

Overall Chemotherapy Treatment Recommendations

Providing precise treatment recommendations for the wide variety of clinical settings of dogs with lymphoma is difficult, especially in light of the plethora of published combination drug protocols (see Table 31-3). Because of the large and ever increasing number of protocols available, several factors should be considered and discussed with caregivers on a case-by-case basis in making the choice of protocol. These factors include the cost, time commitment, efficacy, toxicity, and experience of the
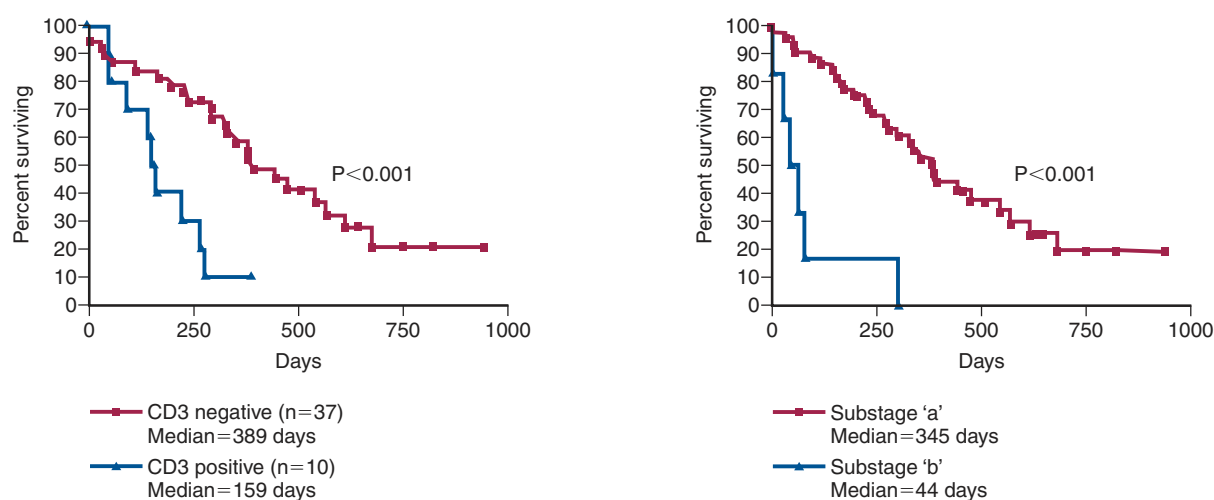
Figure 31-9

A, Kaplan-Meier curve showing survival duration estimates for a group of 55 dogs with lymphoma that were treated with an identical CHOP-based combination chemotherapy protocol. Dogs with CD3-immunoreactive (T-cell) lymphoma had significantly shorter survival durations. **B,** Kaplan-Meier curve showing survival duration estimates for a group of 55 dogs with lymphoma that were treated with an identical CHOP-based combination chemotherapy protocol at the University of Wisconsin. Dogs with substage *b* disease (i.e., clinically ill) had significantly shorter survival durations. *(From Vail DM: Hematopoietic tumors. In Ettinger SJ, Feldman EC, editors: Textbook of veterinary internal medicine, ed 6, St Louis, 2005, Elsevier.)*

clinician with the protocols in question. With the increased availability of generic drugs, protocols are becoming affordable to a larger segment of veterinary clients. In general, more complex combination chemotherapy protocols are more expensive, more time-consuming (i.e., requiring repeated office visits and closer monitoring), and more likely to result in toxicity than simpler, single agent protocols. However, as a general rule, more complex combination protocols result in longer remission and survival durations than single agent protocols.

The earliest treatment protocol used in veterinary patients was a non-doxorubicin-based combination chemotherapy (i.e., cyclophosphamide, vincristine, and prednisone [CVP]), a relatively simple, easy protocol that is well-tolerated and results in a 60% to 70% CR rate and a median survival time of 6 to 7 months.[187,188] However, it has been clearly established that the standard of care combination protocols used in dogs with lymphoma include doxorubicin, and all are essentially variations on the CHOP protocols previously discussed and listed in Table 31-3. Although many of these CHOP protocols include L-asparaginase, which is added either at initiation or at varying times throughout the protocol, several studies have confirmed that the addition of L-asparaginase in induction protocols does not result in clinically relevant increases in remission rate, speed of attaining remission, or first-remission duration; therefore its use is best reserved for rescue situations (discussed later).[128,190,219,220] Regardless of the veterinary

CHOP-based combination protocol used, they all generally result in an 80% to 90% CR rate with median survival times of 12 months. About 25% of dogs are long-term survivors (longer than 2 years), and some are cured. The CHOP protocol used by the authors at the time of publication (Box 31-3) generally is well tolerated by dogs. This protocol does not have a maintenance therapy arm, and all treatments stop at 19 weeks if the animal is in complete remission.

If client factors or other considerations preclude a CHOP-based protocol, single agent doxorubicin can be offered as an alternative, with the patient receiving five doxorubicin treatments (30 mg/m^2 given intravenously every 3 weeks). The expected complete response rate will range from 50% to 75% with an anticipated median survival time of 6 to 8 months.[128,188,189,197]

If financial or other client concerns preclude the use of more aggressive systemic chemotherapy, prednisone therapy alone (2 mg/kg given orally daily) often results in a short-lived remission of approximately 1 to 2 months. In these cases, it is important to inform clients that should they decide to pursue more aggressive therapy later, dogs with previous prednisone therapy are more likely to develop multiple drug resistance (MDR) while receiving single agent prednisone and to have shorter remission and survival durations with subsequent combination protocols. This is especially true after long-term prednisone therapy and in dogs that have experienced a recurrence while receiving prednisone.[195,221] Therefore, the earlier a client

Box 31-3	Current Canine Lymphoma Protocol (L-VCA–Short)

Week 1 Vincristine, 0.7 mg/m², IV
   Prednisone, 2 mg/kg, PO, SID
Week 2 Cyclophosphamide, 250 mg/m², IV*
   Prednisone, 1.5 mg/kg, PO, SID
Week 3 Vincristine, 0.7 mg/m², IV
   Prednisone, 1 mg/kg, PO, SID
Week 4 Doxorubicin, 30 mg/m², IV†
   Prednisone, 0.5 mg/kg, PO, SID
Week 6 Vincristine, 0.7 mg/m², IV
Week 7 Cyclophosphamide, 250 mg/m², IV*
Week 8 Vincristine, 0.7 mg/m², IV
Week 9 Doxorubicin, 30 mg/m², IV†
Week 11 Vincristine, 0.7 mg/m², IV
Week 12 Cyclophosphamide, 250 mg/m², IV*
Week 13 Vincristine, 0.7 mg/m², IV
Week 14 Doxorubicin, 30 mg/m², IV†
Week 16 Vincristine, 0.7 mg/m², IV
Week 17 Cyclophosphamide, 250 mg/m², IV*
Week 18 Vincristine, 0.7 mg/m², IV
Week 19 Doxorubicin, 30 mg/m², IV†

Notes

1. All treatments are discontinued after week 19 if patient is in complete remission (CR).
2. A complete blood count (CBC) should be obtained before each round of chemotherapy. If the neutrophil count is below 2000, the practitioner should wait 5 to 7 days and then repeat the CBC.
3. If the patient develops sterile hemorrhagic cystitis while taking cyclophosphamide, the drug should be discontinued and chlorambucil (1.4 mg/kg, PO) should be given for subsequent scheduled cyclophosphamide injections.
4. For acute lymphoblastic leukemia (ALL), L-asparaginase (400 IU/kg, IM) should be administered with each vincristine injection until CR is achieved.

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Furosemide (1 mg/kg) is given intravenously concurrently with cyclophosphamide to reduce the incidence of sterile hemorrhagic cystitis.
†*In dogs that weigh less than 15 kg, a doxorubicin dose of 1 mg/kg is substituted for 30 mg/m².*
IV, Intravenously; PO, orally; SID, once a day; IM, intramuscularly.

opts for more aggressive therapy, the more likely it is that a durable response will result.

A CBC should be performed before each chemotherapy treatment. A neutrophil count of at least 2000/μl and a platelet count of 50,000/μl should be present before the chemotherapeutic drugs are administered. If the neutrophil count is below 2000/μl, it is best to wait 5 to 7 days and repeat the CBC. If the count has risen above 2000 cells/μl, the drug can be safely administered. A caveat to these restrictions: In dogs presented before initiation of chemotherapy that have low neutrophil and platelet counts because of bone marrow effacement, myelosuppressive chemotherapy is instituted in the face of cytopenias to "open" the bone marrow and allow counts to normalize.

With regard to breeds likely to have MDR-1 gene mutations (e.g., collies; see Chapter 11) and which therefore are at risk for serious, unexpected chemotherapy toxicity, the authors initiate a CHOP protocol out of sequence, beginning with non-MDR-1–associated drugs, such as cyclophosphamide. This ensures treatment of the lymphoma while allowing sufficient time for analysis of MDR-1 gene mutations (see Chapter 11) before MDR-1–associated drugs are initiated.

Reinduction and Rescue Chemotherapy

Eventually, most dogs that achieve a remission relapse or experience a recrudescence of lymphoma. This usually represents the emergence of tumor clones that are inherently more resistant to chemotherapy than the original tumor; so-called MDR clones that either were initially drug resistant or became so after exposure to selected chemotherapeutic agents.[175,222-224] Evidence suggests that in recurrent lymphoma in dogs, tumor cells are more likely to express the MDR-1 gene that encodes the protein transmembrane drug pump often associated with multiple drug resistance.[174,175,224] Other causes of relapse after chemotherapy include inadequate dosing and frequency of administration of chemotherapy and failure to achieve high concentrations of chemotherapeutic drugs in certain sites, such as the central nervous system.

At the first recurrence of lymphoma, reinduction should be attempted first by reintroducing the induction protocol that was successful initially. Special attention must be given to the cumulative dose of doxorubicin that will result from reinduction; also, a baseline cardiac assessment, the use of cardioprotectants, alternative drug choices, and client education should all be considered. In general, the likelihood of a response and the length of the reinduction are half those seen in the initial therapy; however, some animals enjoy long-term reinductions, especially if the patient had completed the initial induction regimen and was off chemotherapy when the relapse occurred. A reinduction rate of nearly 90% can be expected in dogs that have completed CHOP-based protocols and then relapse while off therapy.[129]

If reinduction fails or the dog does not respond to the initial induction, use of so-called rescue agents or rescue protocols can be attempted. These are drugs or

drug combinations that typically are not found in the standard CHOP protocol and are withheld for use in cases of drug resistance. The most common rescue protocols most commonly used in dogs include single agent or combination use of actinomycin D, mitoxantrone, doxorubicin (if doxorubicin was not part of the original induction protocol), a doxorubicin/dacarbazine combination, lomustine (CCNU), L-asparaginase, and the combination mechlorethamine, Vincristine (oncovin) procarbazine, and prednisone (MOPP).[176,206,209-211,225-229] Overall rescue response rates of 40% to 50% are reported, but these responses usually are not durable, and median response times of 1.5 to 2.5 months are typical. A small number of animals will enjoy longer rescue durations. Table 31-4 presents a summary of canine rescue protocols and published results.

Strategies to Enhance the Effectiveness of Chemotherapy in Lymphoma

Despite the plethora of published chemotherapy protocols for dogs with lymphoma, it appears that veterinary medicine has achieved about as much as it can from the currently available chemotherapeutic drugs in standard settings, because no dramatic improvement has been made in the 12-month median survival "wall" and the 25% 2-year survival rate. Advances in remission and survival durations await the development of new methods of delivering or targeting old chemotherapeutic drugs and the development of new generation

chemotherapeutics or novel nonchemotherapeutic treatment modalities. Mechanisms of avoiding or abrogating MDR, enhancing tumor *apoptosis* (programmed cell death), and targeting treatments with immunoconjugates (i.e., antibody-directed therapies), as well as novel immunomodulatory therapies, are all active areas of investigation in both human and veterinary medicine.

Mechanisms of drug resistance

Drug resistance can develop in cancer patients after exposure to selected chemotherapeutic agents and often is associated with expression of P-glycoprotein (see Chapter 11). P-glycoprotein acts as a drug efflux pump that actively extrudes drugs from tumor cells, preventing a cytotoxic drug from reaching the cellular site of action. MDR and P-glycoprotein are controlled by the MDR-1 gene.[222,223,230] MDR has been reported in canine lymphoma after treatment with chemotherapy.[174,224,231] In one study, expression levels of messenger ribonucleic acid (mRNA) that encodes the canine MDR-1 gene was characterized in canine cell lines and lymphomas.[231] Although expression of MDR-1 mRNA correlated with *in vitro* drug sensitivity, it did not correlate with *in vivo* doxorubicin sensitivity in dogs with lymphoma in this study.

Altering drug pharmacokinetics

Methods of increasing the time that tumor cells are exposed to chemotherapeutic drugs theoretically should enhance tumor killing. These methods could

TABLE 31-4	Summary of Response to Rescue Protocols					
Protocol	Number of Animals	Overall Response (%)	Complete Response (%)	Median Response Duration (Days)	Median Duration of Complete Response (Days)	References
Actinomycin-D	12	83	42	42	63	209
Actinomycin-D	25	0	0	0	0	210
Mitoxantrone	44	41	30	Not reported (NR)	127	211
Doxorubicin	12	42	33	145	152	225
Doxorubicin-dacarbazine	15	53	33	<42	NR	226
Mechlorethamine, vincristine, procarbazine, prednisone (MOPP)	117	65	31	61	63	227
Cisplatin-cytosine arabinoside	10	30	10	56	NR	228
Mitoxantrone	15	47	47	NR	84	176
VP-16 (etoposide)	13	15	7	—	—	146, 229
Lomustine (CCNU)	43	27	7	86	110	218

include long-term continuous infusions (impractical in many veterinary situations), increasing the frequency of treatments, or enhancing the drugs' circulation time. In one study, dogs with lymphoma received doxorubicin weekly rather than every 3 weeks in an attempt to reduce cardiac toxicity and increase the duration of drug exposure.[232] No benefit was noted, and in fact, remission rates were inferior. Some studies have evaluated pegylated long-circulating doxorubicin-containing liposome drug delivery systems in dogs with lymphoma.[233,234] Although efficacy was established, no enhancement of remission or survival durations over equivalent doses of native doxorubicin were observed.

Treatment Approaches Using Immunologic or Biologic Agents

Results to date for immunologic procedures and biologic agents have been mixed. A study using the chemical immunomodulator levamisole in combination with chemotherapy proved to be unrewarding in dogs with lymphoma.[235] Autologous vaccines have been shown to have some positive effects in dogs when combined with chemotherapy. A tumor vaccine extract using killed lymphoma cells combined with Freund's adjuvant was administered to dogs after remission induction with combination chemotherapy. The median survival time was 336 days (11 dogs) for the vaccine-treated group versus 196 days (nine dogs) for the group that received chemotherapy alone.[236] A subsequent study by the same investigators reported that the prolonged survival was due to the Freund's adjuvant.[237]

Intralymphatic (IL) administration of an autologous killed lymphoma tumor cell vaccine has been done in dogs in which remission was achieved with a combination chemotherapy protocol. In a study that compared 28 dogs that received chemotherapy and then IL vaccination with 30 dogs that received chemotherapy alone, the median remission times were 98 and 28 days, respectively (p < 0.024).[238] Unfortunately, the survival times for the two groups were not significantly different (305 days for chemotherapy plus IL vaccine and 184 days for chemotherapy alone). Dogs that responded had significant increases in specific antibody to lymphoma antigens compared to those that did not respond.

Another immunotherapy approach involved MAb-231, a murine-derived anticanine monoclonal antibody (IgG2a). This antibody mediates antibody-dependent cellular cytotoxicity (ADDC) and complement-mediated cellular cytotoxicity (CMCC),[239,240] and it prevented outgrowth of canine lymphoma xenografts in nude mice.[241] In a noncontrolled clinical study, 215 dogs were treated with chemotherapy (L-asparaginase, vincristine, cyclophosphamide, and doxorubicin).[242] After two cycles of chemotherapy, 174 dogs had achieved complete remission and were treated with an intravenous infusion of MAb-231 daily for 5 days. The median survival time of the dogs treated with MAb-231 was 493 days. The 2-year survival rate was 15.6%. The median number of chemotherapy cycles in the first year was three, and the median number of MAb-231 cycles was 1.5. The MAb-231 antibody went off the commercial market in the mid-1990s. Definitive randomized trials to determine its effectiveness are still lacking.

Surgery

Most dogs with lymphoma have the multicentric form and need systemic chemotherapy for effective treatment of the disease. However, surgery has been used to treat solitary lymphoma (early stage I) or solitary extranodal disease. In such cases, careful staging is necessary to rule out multicentric involvement before the local disease is treated.

The benefit of surgical removal of the spleen in dogs with massive splenomegaly remains unclear.[243,244] In a published report, 16 dogs with lymphoma underwent splenectomy for a massive spleen and subsequently were treated with chemotherapy. Within 6 weeks of splenectomy, five of the 16 dogs died of disseminated intravascular coagulation (DIC) and sepsis. The remaining 11 dogs had a complete response rate of 66%, and seven of these, which were followed until their death, had a median survival time of 14 months. Splenectomy should be considered only if the lymphoma is in remission in other sites and if the splenic enlargement is caused by lymphoma that is not responsive to chemotherapy. In dogs with lymphoma, splenectomy also can be considered as a treatment for uncontrolled hemolytic anemia and persistent thrombocytopenia.

Radiation Therapy

The role of radiotherapy for the management of lymphoma in dogs currently is under investigation. The use of whole body irradiation without bone marrow transplantation has yielded poor results. However, radiation therapy may be indicated in selected cases.[245-248] The indications are:

- Local stage I or stage II disease (i.e., nasal lymphoma, CNS lymphoma)
- Palliation of local disease (e.g., mandibular lymphadenopathy, rectal lymphoma, mediastinal lymphoma accompanied by precaval syndrome, localized bone involvement)
- Whole body irradiation combined with bone marrow or stem cell transplantation
- Staged half-body irradiation after chemotherapy-induced remission

The use of staged half-body irradiation after achieving remission with induction chemotherapy has undergone

preliminary investigation as a form of consolidation or maintenance.[247,248] In these investigations, radiation therapy is delivered either to the cranial or the caudal half of the dog's body in two consecutive 4 Gray daily fractions; then, after a rest of 3 or 4 weeks, the other half of the body is irradiated in a similar fashion. Although these preliminary investigations were not randomized, their results suggest that radiation therapy, either after completion of chemotherapy or sandwiched between chemotherapy sessions, when dogs are in either complete or partial remission, is safe and should be investigated more extensively to determine whether a significant therapeutic gain could be realized.

Nonmulticentric Lymphoma

In general, the veterinary literature offers little information on the treatment of the various extranodal forms of lymphoma in dogs, therefore the ability to predict outcome is limited. The authors recommend that, after extensive staging, local therapies (e.g., surgery, local radiation therapy) should be used in cases in which disease is localized to a solitary site. If multiple extranodal sites are involved or if they are part of a more generalized process, systemic chemotherapy should be given.

Alimentary lymphoma
Most dogs with alimentary lymphomas have diffuse involvement of the intestinal tract. Involvement of local lymph nodes and the liver is common. Chemotherapy in dogs with diffuse disease has been reported to be unrewarding for the most part.[67] However, in the authors' experience, more aggressive, CHOP-based protocols (which are used extensively for multicentric lymphoma in dogs) have resulted in several cases of durable remission for alimentary lymphoma. Solitary alimentary lymphomas are rare in the dog, but if the tumor is localized and can be removed surgically, the results with or without follow-up chemotherapy can be encouraging.

Primary central nervous system lymphoma
Most CNS lymphoma in dogs results from metastasis of multicentric lymphoma. However, primary central nervous system lymphoma (PCNSL) has been reported.[131,132] If tumors are localized, local radiation therapy should be considered. Few studies have reported on the use of chemotherapy. In one study, cytosine arabinoside (Ara-C) at a dosage of 20 mg/m^2 was given intrathecally by bolus injection after withdrawal of an equal volume of CSF.[131] The dose was diluted in 2 to 4 ml of lactated Ringer's solution and was injected twice weekly for a total of six treatments. This treatment was combined with systemic chemotherapy and CNS irradiation. Overall, the response rates were low and of short duration (several weeks to months).

Cutaneous lymphoma
The treatment of cutaneous lymphoma depends on the extent of disease. Solitary lesions may be treated with surgical excision or radiation therapy. Fractionated radiation therapy (to a total dose of 30 to 45 Gy) has been associated with long-term control.[80] Diffuse non-T-cell lymphoma is best managed with combination chemotherapy, although the response rate is less than in multicentric lymphoma. In one study, investigators reported that combination chemotherapy with cyclophosphamide, vincristine (Oncovin), cytosine arabinoside, and prednisone (COAP) induced long-term remission in some cases.[249] Five of six dogs with diffuse non-T-cell cutaneous lymphoma attained a complete or partial remission, with a median remission duration of longer than 250 days and a median survival of longer than 399 days.[249]

Retinoids, such as isotretinoin (Accutane) and etretinate (Tegison), have been used successfully in canine and human T-cell cutaneous lymphoma.[82,250] In one study, 12 dogs with cutaneous lymphoma were treated with isotretinoin (3 to 4 mg/kg given orally daily), and two were treated with etretinate (1.25 to 1.45 mg/kg given orally daily, continuously). Eleven of these 14 dogs had T-cell cutaneous lymphoma, and six of the 14 dogs achieved remission. In another study, four dogs with T-cell lymphoma were treated successfully with isotretinoin for 13, 11, 10, and 5 months.[82]

In the authors' experience, retinoid treatment must be given for at least 2 months to note a response. Polyethylene glycol (PEG)-L-asparaginase (30 mg/kg given intramuscularly weekly) induces responses in dogs with cutaneous T-cell lymphomas, although remissions are not durable, and no cures have been noted.[251] Prednisone may also be necessary to control pruritus. In the authors' experience, pegylated-liposomal doxorubicin (Doxil) has produced remissions in approximately 40% of cases. Although most of these were short-lived responses, remissions of 1 year or longer occasionally have occurred.

A preliminary abstract reported activity for oral CCNU with cutaneous lymphoma in dogs.[252] All seven dogs (five with mycosis fungoides [MF] and two with nonepitheliotropic disease) that were treated with CCNU (50 mg/m^2 given orally every 3 weeks) achieved a complete response, and two of those responses were relatively durable (7 and 15 months). A larger cohort needs to be treated before definitive response rates and durations can be given.

Topical chemotherapy is another strategy for treating cutaneous T-cell lymphoma, although it is rarely used in veterinary medicine because of "patient compliance" problems. Mechlorethamine (Mustargen) can be applied topically as an aqueous solution or an ointment base. The aqueous solution is prepared by combining 10 mg of mechlorethamine with 50 ml of tap water.

The ointment is prepared by mixing 90 mg of mechlorethamine with 10 ml of absolute alcohol and further combining enough xipamide (Aquaphor) to prepare 900 g of ointment. Hair must be removed before application. Gloves must be used when the drug is applied, because mechlorethamine is carcinogenic and can cause contact hypersensitivity in humans. The response to therapy varies, and the treatment often is only palliative.[253]

Prognosis

The prognosis for canine lymphoma varies and depends on a number of factors, such as the location of disease, the extent of disease (the clinical stage), the presence or absence of clinical signs (the substage), the histologic grade, the immunophenotype (T cell or B cell), exposure to previous chemotherapy or corticosteroids and subsequent development of MDR (see Chapter 11), altered cell death processes (apoptosis), the proliferation rate of the tumor, the presence of concurrent medical problems or paraneoplastic conditions (e.g., hypercalcemia, weight loss, and liver insufficiency), and possibly gender.* Although canine lymphoma is rarely curable (fewer than 10% of cases), complete responses and a good quality of life during extended remissions and survival are typical.

Factors that have been shown to influence survival are shown in Table 31-5. The two factors most consistently identified as being prognostically important in dogs with lymphoma are the immunophenotype and WHO substage (see Figure 31-9). Many reports have confirmed that dogs with CD3-immunoreactive tumors (i.e., T-cell derivation) are associated with significantly shorter remission and survival durations.[†] This holds true primarily for dogs with multicentric lymphoma, because the immunophenotype of solitary or extranodal forms of lymphoma has not been thoroughly investigated with respect to the prognosis. In addition, it has been shown that dogs with B-cell lymphomas that express lower than normal levels of B5 antigen (expressed in 95% of nonneoplastic lymphocytes) also have shorter remission and survival durations.[75] Dogs with WHO substage *b* disease (i.e., clinically ill) also do poorly compared to dogs with substage *a* disease (see Figure 31-9).[‡] Dogs with stage I or stage II disease have a better prognosis than dogs with more advanced disease (stage III, IV, or V).[187,188] In some studies, an elevated serum calcium (over 11.9 mg/dl) has been shown to be a negative prognostic factor[72,257]; however,

this rarely holds true with multivariate analysis because hypercalcemia is associated with the T-cell phenotype.

The histologic grade (subtype) has been found to influence the prognosis in some studies; however, our ability to predict outcome based on subtype is still quite limited. Dogs with lymphoma classified as intermediate- or high-grade (large cell, centroblastic, and immunoblastic) tend to respond to chemotherapy but can relapse early. Dogs with low-grade lymphomas (small lymphocytic or centrocytic) have a lower response rate to chemotherapy, yet have a survival advantage over dogs with intermediate- or high-grade lymphomas (Figure 31-10) in that the course of disease may be more indolent.[113] Using the Working Formulation, dogs with low-grade lymphomas have a survival advantage compared to dogs with intermediate- or high-grade tumors.[78]

Recently, proliferative assays (e.g., analysis of bromodeoxyuridine [BrdU] uptake, Ki-67 antibody reactivity, and AgNOR indices) to measure the proliferative activity of tumor cells have been shown to provide significant prognostic information in dogs treated with combination chemotherapy.[60,163,254,258-260] However, the results of different studies are contradictory. In two trials, dogs that had tumors with short doubling times, high AgNOR frequencies, or high Ki-67 immunoreactivity had a better prognosis than dogs with long doubling times or low AgNOR frequencies.[60,163] In other trials, the low-proliferating tumor groups were associated with a better prognosis.[254,259] In one trial, the proportion of tumor cells undergoing apoptosis was modestly predictive of remission duration.[163]

The anatomic site of disease also has considerable prognostic importance. Primary diffuse cutaneous, diffuse gastrointestinal, hepatosplenic, and primary CNS lymphomas tend to be associated with a poor prognosis. Cutaneous lymphoma tends to progress slowly, and in our experience the responses to systemic chemotherapy are less durable. Localized lymphomas in the skin can be managed with radiation therapy or surgery or both, and these tumors have a better prognosis. In some dogs with lymphoma, significant involvement in the bone marrow may be present (i.e., tumor cells comprise more than 50% of all nucleated cells) and circulating malignant lymphocytes may be present in the peripheral blood. These dogs tend to have an overall poor prognosis. In some cases it is difficult to determine whether the disease arises from the bone marrow (e.g., acute lymphoblastic leukemia [ALL]) or is a diffuse lymphoma with extensive involvement in the marrow. Immunophenotyping is helpful in these cases, because the tumor cells in ALL typically are CD34 positive.

Gender has been shown to influence the prognosis in some studies.[59,191] Neutered females tend to have a better prognosis. Males may have a higher incidence of

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*References 59, 60, 61, 63, 72, 74, 75, 113, 174, 187-189, 224, and 254-256.
[†]References 60, 72, 75, 112, 113, and 256.
[‡]References 59, 60, 72, 112, 128, and 255.

TABLE 31-5 Prognostic Factors for Lymphoma in Dogs

Factor	Strong Association	Modest Association*	Comments	References
World Health Organization (WHO) clinical stage		X	Stage I/II: Favorable. Stage V with significant bone marrow involvement: Unfavorable.	187, 188, 255
WHO clinical substage	X		Substage-b (clinically ill) associated with reduced survival.	59, 60, 72, 113, 128, 255
Histopathology		X	High/medium grade associated with high response rate but reduced survival.	113, 114
Immunophenotype	X		T-cell phenotype associated with reduced survival.	60, 72, 75, 112, 113
Hypercalcemia		X	Negative factor if associated with T-cell subtype and reduced renal function.	72, 257
Gender		X	Some studies suggest females have a favorable prognosis.	59, 191
Measures of proliferation		X	Reports are contradictory.	60, 163, 254, 258, 259
Prolonged steroid pretreatment	X		Most reports suggest that previous steroid use shortens response duration, but critical length of exposure is unknown.	195, 221
P-glycoprotein expression		X	May be associated with poor response rate and shortened remission.	174, 175, 224
Craniomediastinal lymphadenopathy	X		Large compilation of cases reports shorter remission and survival.	63
Anatomic location	X		Leukemia, diffuse cutaneous and alimentary, and hepatosplenic forms associated with unfavorable prognosis.	67, 90-92
Circulating enzyme markers		X	Levels of both glutathione–S–transferase and thymidine kinase have been reported to predict outcome, but results need confirmation.	261, 262
Serum VEGF levels		X	Small study suggests that pretreatment levels are predictive of remission duration.	263
History of chronic inflammatory disease		X	Predicts likelihood of early relapse.	264

*Requires further investigation.
VEGF, Vascular endothelial growth factor.

Figure 31-10

Kaplan-Meier curves showing time to relapse adjusted for clinical stage and immunophenotype among dogs treated for lymphoma classified according to the Kiel system as low grade (n = 17) *(blue line)* or high grade (n = 51) *(red line)*. *(From Teske E, van Heerde P, Rutteman GR et al: J Am Vet Med Assoc 205:1722-1728, 1994.)*

the T-cell phenotype, which may account for the poorer prognosis.[60]

Other reported potential biomarkers of the prognosis include circulating levels of glutathione-S-transferase, thymidine kinase, and vascular endothelial growth factor (VEGF).[261-263] One report suggests that a history of chronic inflammatory disease of several types predicts a likelihood of early relapse.[264] These putative prognostic indicators require confirmation in larger trials.

LYMPHOID LEUKEMIA

Lymphoid leukemia is the proliferation of neoplastic lymphocytes, which usually originate in the bone marrow but occasionally may originate in the spleen. The neoplastic cells may or may not be circulating in the peripheral blood.

Incidence and Risk Factors

Lymphocytic leukemia is more common than nonlymphocytic leukemia and other myeloproliferative disorders (MPDs). The true incidence is not known. In a series of 30 cases of ALL, German shepherds accounted for 27% of the cases, and the male to female (M:F) ratio was 3:2.[265] In this study, the median age was 5.5 years (range, 1 to 12 years), and eight dogs were younger than 4 years of age. Recently, ALL was reported in a 12-week-old greyhound.[266] Well-differentiated or chronic lymphocytic leukemia (CLL) is seen less frequently than ALL but more commonly than MPD. The median age is

10 to 12 years. The M:F ratio has been reported as 1.8:1 (22 dogs) and approximately 2.3:1 in 15 dogs with granular lymphocytic (GL) T-cell CLL.[267] In a large study of 73 dogs with CLL, no gender predilection was found in dogs with B-cell or non-GL CLL; female dogs were overrepresented among dogs with GL CLL, (M:F ratio, 1:1.7).[268,269]

Etiology

As with lymphoma, the etiology of lymphoid leukemia is unknown. Retroviruses have been implicated in diverse animal species such as cats, cattle, fish, snakes, birds, rodents, and nonhuman primates. A retroviral cause in dogs has not been proved. However, a retrovirus with morphology typical of lentiviruses has been isolated from mononuclear cells obtained from the peripheral blood of a dog with ALL.[270]

In humans, acute leukemia has been associated with exposure to radiation, benzene, phenylbutazone, and antineoplastic agents.[271-276] The alkylating agents can cause chromosomal damage and are clearly carcinogenic.[276] Human T-lymphotropic virus type 1 (HTLV-1) is a proven cause of leukemia in a large cohort of human patients from the southern islands of Japan.[277,278] The etiology of CLL is less clear, but genetic factors likely are important. Extrapolation of predisposing factors across species is not warranted, and the etiologic factors for dogs may be quite different from those for humans, given the difference in the predominant immunophenotype of the neoplastic cells (discussed later).

Pathology and Natural Behavior

In ALL, the blast cells infiltrate the bone marrow, and the result is variable degrees of anemia, thrombocytopenia, and neutropenia. Infiltration of the spleen and liver is common, and extramedullary sites (e.g., the nervous system, bone, and GI tract) also may be involved. Some animals may have lymph node involvement and develop generalized lymphadenopathy.[265]

The lymphocytes of CLL are virtually indistinguishable morphologically from normal small lymphocytes, and they have a low proliferation rate. The accumulation of lymphocytes likely results from their prolonged life span. In B-cell CLL, the marrow is infiltrated with mature lymphocytes, and the extent of infiltration is less than that seen with ALL or MPD. The neoplastic cells in GL T-cell CLL originate in the spleen, and bone marrow involvement may or may not occur.[173] Dogs with CLL tend to have a mild anemia, and granulocytes and platelets are only mildly reduced. Splenomegaly is common, and lymph nodes can be slightly to moderately enlarged.[269]

Despite the well-differentiated appearance of the lymphocytes in CLL, these cells may function abnormally.

Paraneoplastic syndromes include monoclonal gammopathies, immune-mediated hemolytic anemia, pure red cell aplasia and, rarely, hypercalcemia.[173,279] Hypercalcemia was reported in a giant schnauzer with B-cell CLL, which is highly unusual given that hypercalcemia is associated with T-cell lymphoproliferative disorders. In one study of 22 dogs with CLL, 68% had monoclonal gammopathies.[269] The immunophenotypes were not reported, but a monoclonal gammopathy is associated with production of immunoglobulins by the leukemic cells (B cells). The immunoglobulin is usually IgM or IgA. The term *macrogammaglobulinemia* is used to describe IgM gammopathy (see Chapter 31, section D). Dogs with CLL and an IgM monoclonal gammopathy are said to have Waldenström's macroglobulinemia. Dogs can also develop hyperviscosity syndrome (see Chapter 31, section D).

Reports of the immunophenotype of neoplastic cells in canine leukemias are increasing in frequency. One consistent finding in acute leukemias (either ALL or acute myeloid leukemia) is the presence of CD34 on the blast cells. This marker is useful for distinguishing ALL (CD34-positive) from CLL and lymphoma with bone marrow infiltration (CD34-negative). In one study of 12 cases of CLL, eight dogs were associated with a CD8-positive T-cell phenotype.[75] In a larger study of 73 cases, only 26% of the cases had a B-cell immunophenotype, whereas 73% were identified as T-cell CLL (CD3-positive). In 54% of these T-cell leukemias, the cells had the morphology of GLs, and most of them were CD8-positive. These findings are difficult to reconcile with the high frequency of monoclonal gammopathies reported in the earlier study. It also should be noted that this differs from human CLLs, which typically are lymphoproliferative diseases of B cells.

History and Clinical Signs

Dogs with ALL usually have a history of anorexia, weight loss, polyuria, polydipsia, and lethargy. Splenomegaly is typical, and other physical abnormalities may include hemorrhages, lymphadenopathy, and hepatomegaly. Anemia, thrombocytopenia, and an elevated white blood cell (WBC) count are common. The anemia may be severe and usually is characterized as normocytic and normochromic (nonregenerative). WBC counts usually are increased despite neutropenia because of an increased number of circulating lymphoblasts (more than 14,000 cells/μl). However, some dogs may be leukopenic.[265] Neoplastic lymphoblasts may infiltrate the bone marrow extensively, resulting in depression of normal hematopoietic elements.

Dogs with CLL often are asymptomatic, although some owners report lethargy and a decreased appetite. Mild lymphadenopathy and splenomegaly may be present. Most dogs tend to be mildly anemic (packed cell volume [PCV] less than 35%) and thrombocytopenic (110,000 to 190,000 platelets/μl). The WBC count usually exceeds 30,000 cells/μl but can vary from normal to more than 100,000 cells/μl, owing to an increase in circulating mature lymphocytes.[269] Lymphocytosis is persistent, and granulocytes usually are present in normal numbers. In some dogs the disease is identified incidentally when the patient undergoes evaluation for an unrelated problem.

Diagnostics and Clinical Staging

The clinician must consider the signalment, history, physical findings, cell morphology, and immunophenotype to diagnose the lymphoid leukemias accurately. A knowledge of the profile of lymphocyte subsets in the peripheral blood of normal dogs is helpful for determining whether a particular subset has expanded. Approximately 80% of circulating lymphocytes in dogs are T cells, and about 15% are B cells. NK cells and double-negative (CD4–CD8–) T cells account for the remaining fraction. In the T-cell fraction, helper T cells (CD4+) outnumber cytotoxic T cells (CD8+).[173]

Lymphocytic leukemia should be a consideration if atypical lymphocytes are in circulation, if the immunophenotype of the lymphocytes in circulation is homogenous, or if a phenotype typically present in low numbers has increased. Differential diagnoses for lymphocytosis include certain infectious diseases (e.g., chronic ehrlichiosis), postvaccinal responses in young dogs, IL-2 administration, and transient physiologic or epinephrine-induced lymphocytosis. Reactive and neoplastic lymphocytoses sometimes are difficult to differentiate. In these cases, PCR assays are used to determine the clonality of the T cell or immunoglobulin receptor genes in a population of lymphocytes (see earlier discussion).[167,169,268]

Infiltration of the bone marrow by neoplastic lymphoid cells is the hallmark of ALL and is seen in most cases of CLL; careful examination of peripheral blood and bone marrow by experienced cytopathologists is essential for establishing a diagnosis of lymphocytic leukemia. If adequate diagnostic bone marrow cannot be obtained by aspiration, a bone marrow core biopsy should be performed. In ALL, lymphoblasts predominate in the bone marrow and also are present in peripheral blood. Infiltration of bone marrow by lymphoblasts is accompanied by a decrease in the granulocytic, erythroid, and megakaryocytic cell lines.

Perhaps the distinguishing feature of lymphoblasts is the nuclear chromatin pattern, which is more condensed than the chromatin in myeloblasts.[280] Lymphoblasts are larger than neutrophils, have a high nucleus-to-cytoplasm ratio, and blue cytoplasm, which in some cases is intensely basophilic (Figure 31-11, *A*). Nucleoli, although present, are less prominent in
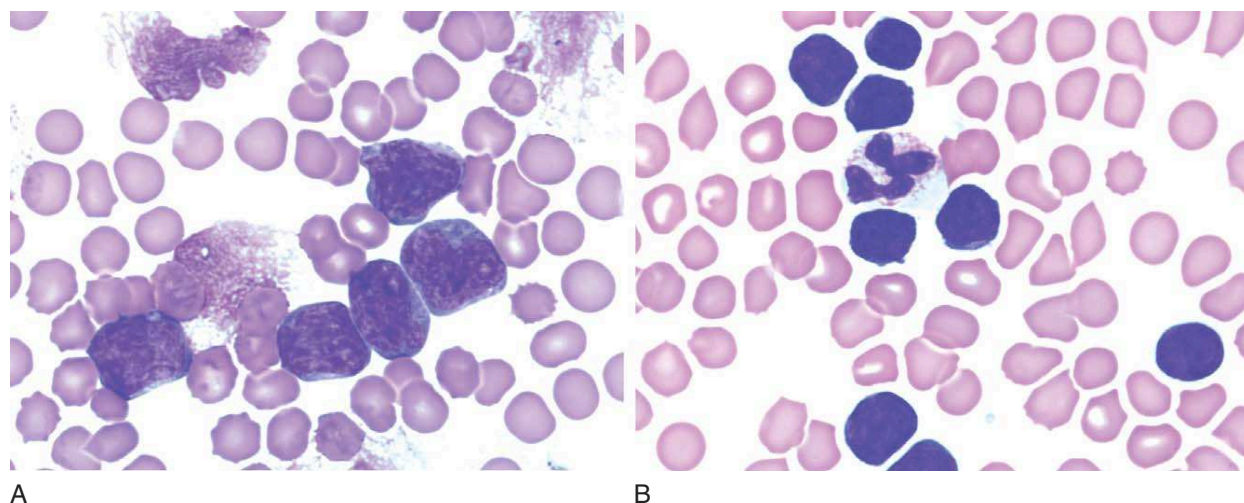
Figure 31-11
A, Peripheral blood from a dog with acute lymphoblastic leukemia (ALL). Note the large lymphoid cells with visible nucleoli. Chromatin from disintegrated cells also is visible. (Wright's stain, ×60 objective.)
B, Peripheral blood from a dog with chronic lymphocytic leukemia (CLL). Note the small lymphocytes of normal morphology (i.e., smaller than the neutrophil). (Wright's stain, ×60 objective.)

lymphoblasts than in myeloblasts. Nevertheless, these cells cannot be easily distinguished from blast cells of other hematopoietic lineages, and lineage-specific markers on the cells must be identified by immunocytochemistry or flow cytometry.[278] The most frequently used markers are CD3 for T cells and CD79a for B cells. As mentioned earlier, CD34 is found on blast cells of both lymphoid and myeloid lineages, and positivity for CD34 helps to confirm leukemia of immature cells (acute leukemia) and to distinguish ALL from stage V lymphoma. Immature and differentiating lymphocytes may stain strongly for alkaline phosphatase activity, which suggests that this cytochemical staining procedure is not specific for myeloid leukemia.[278,280,281]

In B-cell and non-GL T-cell CLL, the lymphocytes are small mature cells (Figure 31-11, *B*) that appear in excessive numbers in bone marrow (30% or more of all nucleated cells) early in the disease.[269] Infiltration becomes more extensive as the disease slowly progresses, and eventually the neoplastic cells replace normal marrow. The lymphoid lineage of the cells in CLL typically is easy to identify, and immunophenotyping is done to determine the lymphocyte subset. In dogs, most cases of CLL are T-cell (CD3+) proliferations. In GL CLL, the neoplastic cells originate in the spleen.[173]

A separate clinical staging system has not been developed for lymphocytic leukemia. Currently, all dogs with leukemia are classified as stage V based on the WHO staging system for lymphoma (see Box 31-1). Although a specific clinical staging system for CLL has

been used in humans, it has not been evaluated in the dog.[282]

Treatment of the acute lymphoblastic leukemia

Similar to other infiltrative bone marrow malignancies, ALL causes morbidity by suppressing bone marrow function. Neutropenia, thrombocytopenia, and anemia may be severe. Therapy must be aggressive; to restore hematopoiesis, a 1.5 to 2 logarithmic reduction in leukemic cell numbers (to less than 100 million cells) must be achieved. Patients need supportive therapy, such as fresh whole blood, broad-spectrum antibiotics, fluid therapy, and nutritional support. Patients must be monitored carefully for bleeding and thrombosis, which may signal the development of DIC.

ALL requires aggressive chemotherapy. Consistently efficacious protocols for ALL have not been developed in veterinary medicine. CHOP-based protocols, similar to those used for lymphoma (see Box 31-3), tend to be used as treatment protocols for dogs with ALL. In one report on the use of vincristine and prednisone in dogs with ALL, 40% of the dogs responded to vincristine and prednisone, 20% with a complete remission and 20% with a partial remission.[265] With the addition of doxorubicin and L-asparaginase, it is anticipated that response rates will increase over those previously reported using vincristine and prednisone alone; however, the relative rarity of ALL limits the ability to identify effective protocols.

Box 31-4	*Treatment of Chronic Lymphocytic Leukemia*

Regimen 1

Chlorambucil, 0.2 mg/kg, PO, days 7-14; *then*
  0.1 mg/kg, PO, continuously *or* 2 mg/m^2, PO, QOD*
Prednisone, 30 mg/m^2, PO, SID, for 7 days; *then*
  20 mg/m^2, PO, SID, for 7 days; *then*
  10 mg/m^2, PO, QOD

Regimen 2

Vincristine, 0.02 mg/kg, IV, weeks 1-3
Chlorambucil, 0.2 mg/kg, PO, SID, for 7 days; *then*
  0.1 mg/kg, PO, SID, continuously *or* 2 mg/m^2,
  PO, QOD*
Prednisone, 30 mg/m^2, PO, SID, for 7 days; *then*
  20 mg/m^2, PO, SID, for 7 days; *then*
  10 mg/m^2, PO, QOD, continuously

If no response, substitute cyclophosphamide, 50 mg/m^2, PO, SID, for 4 days; repeat weekly.

PO, Orally; QOD, every other day; SID, once a day; IV, intravenously.

Treatment of chronic lymphocytic leukemia

Because of the indolent nature of CLL in many animals, the question of whether all dogs with the disease should be treated is controversial.[283,284] Most oncologists recommend observation over active therapy when the discovery of CLL is incidental, when no physical or clinical signs are present, and when no significant hematologic abnormalities are identified. Therapy should be instituted if the animal is anemic or thrombocytopenic, is showing evidence of significant lymphadenopathy or hepatosplenomegaly, or has an excessively high WBC count (over 60,000 lymphocytes/μl) (Box 31-4). The most effective drug evaluated thus far is chlorambucil.[269] Chlorambucil is administered at a dosage of 0.2 mg/kg or 6 mg/m^2 given orally once daily for 7 to 14 days. The dosage then can be reduced to 0.1 mg/kg or 3 mg/m^2 given orally once daily. For long-term maintenance, a dosage of 2 mg/m^2 given orally every other day can be used. The dosage is adjusted according to the clinical response and bone marrow tolerance. Chlorambucil should be administered without food to increase the rate of absorption.[285]

Corticosteroids are lymphocytolytic and lead to cell death by apoptosis. Studies in humans have shown that the antitumor activity of chlorambucil combined with prednisone is better than that of chlorambucil alone.[286] When the bone marrow is heavily infiltrated with CLL cells, and neutropenia, thrombocytopenia, and anemia occur, use of a more aggressive alkylating agent (usually cyclophosphamide [250 mg/m^2 given once]) when initiating oral chlorambucil and prednisone therapy can be considered to increase the speed of initial remission; however, this modification of the protocol has not been scrutinized in clinical trials. If chlorambucil or cyclophosphamide fails, the choice of treatment is combination chemotherapy similar to that presented in Box 31-3. The treatment of CLL is primarily palliative, and complete remissions are rare. Because of the indolent nature of this disease, however, survival times have been in the range of 1 to 3 years with a good quality of life.[269,284]

In humans, splenectomy has been shown to increase survival significantly in individuals with aggressive forms of CLL; however, splenectomy in dogs with CLL has not been evaluated.[287] The phenotypic expression of CLL usually is stable over months to years. However, the disease may evolve into an acute phase, and some dogs develop a rapidly progressive, pleomorphic (immunoblast) lymphoma.[269] In humans, this is called *Richter's syndrome*.[288] The prognosis for response to treatment is poor for this form.

Prognosis

In general, the prognosis for ALL in the dog is very poor. In a study of 21 dogs treated with vincristine and prednisone, the dogs achieving complete or partial remission (29%) had a median survival time of 120 days, and few dogs survived longer than 8 months with that protocol.[265] In one case report, a dog with ALL was treated with an infusion of a large volume of fresh canine plasma and whole blood, and a complete remission was maintained for 19 months without additional therapy. This is a very unusual response, which indicates that normal blood contains some antileukemic factor or factors.[289]

As stated before, CLL is a slowly progressive disease, and some animals do not require therapy. One dog was observed for almost 2 years without treatment.[283] For dogs that are treated, normalization of WBC counts can be expected in 70% of cases. In one report of 17 dogs treated with vincristine, chlorambucil, and prednisone, the median survival time was approximately 12 months, with an expected 30% survival at 2 years.[269] In other studies, dogs treated intermittently with chlorambucil and prednisone have had remissions of 10 to 30 months.[284,290,291]

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SECTION B
Feline Lymphoma and Leukemia

David M. Vail

LYMPHOMA

The lymphomas (malignant lymphoma and lymphosarcoma) are a diverse group of neoplasms that have in common their origin from lymphoreticular cells. They usually arise in lymphoid tissues, such as lymph nodes, spleen, and bone marrow; however, they may arise in almost any tissue in the body. Lymphoma is one of the most common neoplasms seen in the cat.

Incidence and Etiology

Viral factors

The feline leukemia virus (FeLV) was the most common cause of hematopoietic tumors in the cat during the so-called FeLV era of the 1960s through the 1980s, when 60% to 70% of lymphoma cases were associated with FeLV antigenemia.[1-7] Several studies have documented the potential molecular means by which FeLV can result in lymphoid neoplasia.[8,9] However, over the past 20 years in North America, a profound change has occurred in the viral status, presentation, signalment, and frequency of anatomic sites in cats with lymphoma, as documented by two large studies of feline lymphoma involving more than 700 cats.[10,11] The change in the epidemiology and characteristics of lymphoma in cats appears to coincide with the widespread integration of clinically relevant FeLV diagnostic assays (indirect immunofluorescence assay) and the affected animal elimination regimens of the late 1970s; it was further enhanced by the appearance of commercially available FeLV vaccines in the late 1980s.

The decline in FeLV-associated lymphoma was mirrored by a decline in the overall prevalence per year of FeLV positivity in cats tested, as characterized by reports from the Tufts Veterinary Diagnostic Laboratory from 1989 to 1997[10,12] and by Louwerens' group,[11] which reported a decline in FeLV association in more than 500 cases of lymphoma in cats brought to the University of California–Davis Veterinary Teaching hospital. In these reports, FeLV antigenicity represented only 14% to 25% of the cats with lymphoma.

The incidence figures for feline lymphoma generated before the use of FeLV vaccination and testing became widespread suggest that lymphoma accounted for 50% to 90% of all hematopoietic tumors in the cat[13,14]; because hematopoietic tumors (lymphoid and myeloid) represent approximately one third of all feline tumors, the estimated incidence of lymphoid neoplasia was 200 per 100,000 cats at risk.[15] In one series of 400 cats with hematopoietic tumors, 61% had lymphoma and 39% had leukemias and MPDs (21% of the MPDs were categorized as undifferentiated leukemias, most likely myeloid in origin).[16] An important fact that Louwerens' study revealed is that despite a sharp drop in FeLV-associated lymphoma, the overall prevalence of lymphoma in cats is increasing.[11] This appears to be due to an increase in the number of affected cats and the relative frequency of the abdominal (particularly the intestinal) anatomic form of lymphoma in the species (Figure 31-12).

As might be expected, along with a shift away from FeLV antigen–associated tumors came a shift away from the traditional signalment and relative frequency of anatomic sites.[10,11] This observation is supported outside North America by the similar signalment and anatomic frequency data obtained in Australia, where FeLV infection is quite rare.[17-20] The median age of approximately 11 years now reported in North America for lymphoma in cats is considerably higher than the median age of 4 to 6 years reported during the FeLV era.[1,3-6,10,11,21] The median age of cats within various anatomic tumor groupings has not changed, and anatomic forms traditionally associated with FeLV, such as the mediastinal form, still occur in younger, FeLV-antigenemic cats. Similarly, the alimentary form occurs most often in older, FeLV-negative cats.[10,11,18-20] Table 31-6 presents an overview of the characteristics of the various anatomic sites of lymphoma in cats. Younger cats with lymphoma tend to be FeLV antigenemic
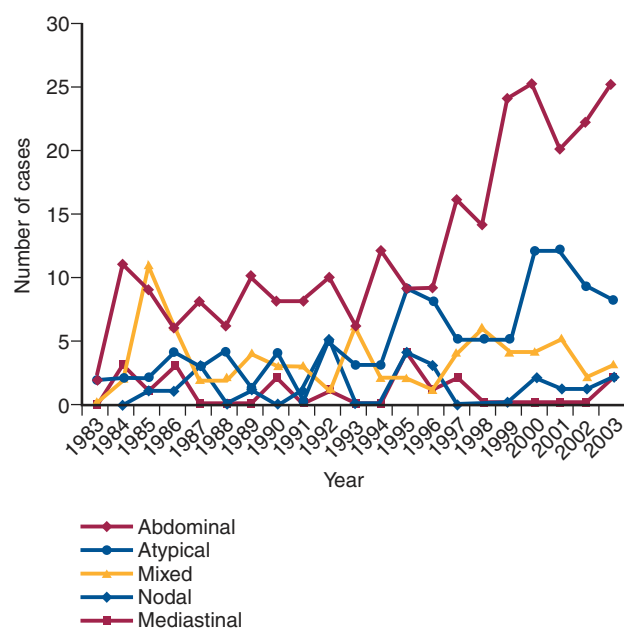
and are more likely to have mediastinal or multicentric lymphoma. Most cats with spinal lymphoma (85% to 90%) also are FeLV antigenemic.[22,23] Older cats usually are FeLV negative and develop alimentary lymphoma.[10,17,19,20,24-27] In a large compilation of Australian cases, male cats and the Siamese/oriental breeds were overrepresented.[17] Similar breed findings have been observed in North America,[11] but similar gender findings have not emerged.[28,29] The Siamese/oriental breeds appear to have a predisposition for the mediastinal form that affects a younger population (median age, 2 years).[11]

Evidence also indicates that feline immunodeficiency virus (FIV) infection can increase the incidence of lymphoma in cats.[30-37] In contrast to FeLV, which plays a direct role in tumorigenesis, FIV appears to have an indirect role, likely secondary to the immunosuppressive affects of the virus.[35] Shelton and colleagues[30] determined that FIV infection alone in cats was associated with a fivefold increased risk for the development of lymphomas. Coinfection with FeLV further potentiates the development of lymphoproliferative disorders. Experimentally, cats infected with FIV have developed lymphoma in the kidney, alimentary tract, and liver and in multicentric sites. FIV-associated lymphoma is more likely to have a B-cell immunophenotype whereas T-cell type is predominantly associated with FeLV.[32,33,35,36,38-41] Some have suggested that FIV infection may be associated more often with alimentary lymphoma of B-cell origin,[40,41] which may be related to chronic dysregulation of the immune system or activation of oncogenic pathways. However, in another large compilation of cases, FIV antigenemia only rarely was associated with alimentary lymphoma.[10,24-27]

Genetic and molecular factors

As discussed in Chapter 31, section A, recent advances in molecular cytogenetics (see Chapter 1, section A), including gene microarray techniques, are being used in investigations of chromosomal aberrations in veterinary species with lymphoma. The previously mentioned predisposition of the oriental cat breeds to the development of lymphoma suggests a genetic predisposition and indicates heritable risks.[11,17] As our knowledge of molecular events and tumorigenesis has expanded, several molecular aberrations have been implicated in various feline tumor types, and some associated with lymphoma have been identified. Altered oncogene/tumor suppressor gene expression, epigenetic changes, signal transduction, and death-pathway alterations are common in human lymphomas and likely are also involved in the cat. N-*ras* aberrations, although rare in cats, have been implicated.[42] Telomerase activity (see Chapter 14, section D) also has been documented in feline lymphoma tissues.[43,44] Other factors implicated in feline lymphoma include alterations in cellular proliferation and in cell cycle and death (apoptosis) pathways, particularly the cyclin-dependent kinase cell cycle regulators and the Bcl-2 family of proapoptotic and antiapoptotic governing molecules, which have been implicated in human NHL.[45,46]

Environmental factors

Evidence that exposure to environmental tobacco smoke (ETS) is a risk factor for lymphoma in humans has prompted investigations in cats. In one report, cats with any exposure to ETS had a relative risk of developing lymphoma of 2.4; the relative risk for cats with 5 or more years of exposure was 3.2.[47]

Immunosuppression

Immune system alterations in the cat, such as those brought on by FIV infection, have been implicated in the development of lymphoma.[30-33,35-37] In a report on 95 feline renal transplant recipients, nearly 10% developed *de novo* malignant lymphoma, which bolsters support for a link to immunosuppression in the species. These findings are similar to those seen in immunosuppressed human organ transplant patients.[48]

TABLE 31-6 General Characteristics of Various Anatomic Forms of Lymphoma in Cats Based on Three Large Compilations

Anatomic Form	FREQUENCY (%)			MEDIAN AGE (YR)			% FeLV ANTIGENEMIC			% B CELL*			% T CELL		
	USA[11] (N=477)	USA[10] (N=145)	AUS[18†] (N=109)	USA[11] (N=477)	USA[10] (N=145)	AUS[18†] (N=118)	USA[11] (N=477)	USA[10] (N=145)	AUS[18†] (N=109)	USA[11] (N=477)	USA[10] (N=145)	AUS[18†] (N=118)	USA[11] (N=477)	USA[10] (N=145)	AUS[18†] (N=109)
Intestinal	39	34.7	48.3	12	12	11.5		5.4				75.4		14	22.8
Abdominal without intestinal[‡]	3	NR	NR	NR	NR	NR		NR				NR		NR	NR
Mediastinal	5.7	11.8	21.2	2	5	5		73				48		85	48
Multicentric abdominal[§]	5	NR	NR	NR	NR	NR		NR				NR		NR	NR
Leukemia	NR	NR	11.8	NR	NR	NR		NR				21.4		NR	78.6
CNS	4.8	NR	NR		NR	NR		NR				NR		NR	NR
Cutaneous	NR	NR	1.7	NR	NR	12.5		NR				NR		NR	NR
Renal	3.1	6.9	28.8	NR	7.5	7.5		25				82.4		0	14.7
Hepatic	NR	5.6	11.9	NR	11	NR		57				42.9		14	57.1
Other extranodal	9.9	13.1	15.3	NR	9.5	11		20				83.3		13	11.1
Pure nodal	4.8[¶]	NR	NR	10	NR	NR		NR				62.8		NR	32.6
Nasal	6.3	9.7	4.2	NR	12	9		0				NR		8	NR
Mixed[‖]	16.4	18.8	NR	10	8	NR		31				NR		37	NR
Overall				11	9.5	9.5	14.5	25.5				70		25.4	26

From Vail DM, Moore AS, Ogilvie GK et al: Feline lymphoma (145 cases): proliferation indices, CD3 immunoreactivity and their association with prognosis in 90 cats receiving therapy, J Vet Intern Med 12:349-354, 1998; Louwerens M, London CA, Pedersen NC et al: Feline lymphoma in the post-feline leukemia virus era, J Vet Intern Med 19:329-335, 2005; and Gabor LJ, Canfield PJ, Malik R: Immunophenotypic and histological characterization of 109 cases of feline lymphosarcoma, Aust Vet J 77:436-441, 1999.

#Includes those reported as "intra-abdominal" when intestinal disease is a documented component.
*If only a T-cell assessment was made, the B-cell frequency is given as not reported (NR) but can be inferred from the frequency of T cells.
†Report does not separate mixed form from pure forms, therefore percentages add up to more than 100.
‡Excluding renal only cases.
§Intestinal and other intra-abdominal organs; does not include cases in which disease was confined to multicentric nodes.
¶Lymphoma-positive nodes were part of the presentation in 24% of all cases.
‖Cases in which more than one category applied and the primary site could not be determined.

FeLV, Feline leukemia virus; CNS, central nervous system.

Chronic inflammation

Although definitive proof is lacking, a growing body of indirect evidence suggests that lymphoma may be associated with a state of chronic inflammation, such as in intestinal and nasal lymphoma. In particular, an association has been suggested between intestinal lymphoma and inflammatory bowel disease[11,49-51]; however, others have not found support for this concept.[52] Additional support for this association is provided by a recent report that suggests that cats with vaccine site–associated sarcoma, a syndrome directly linked to inflammation, are also at risk for the development of lymphoma.[53]

Diet and intestinal lymphoma

Although no direct evidence exists, a link between diet and the development of intestinal lymphoma in cats has been suggested.[11] This association is supported by the relative and absolute increase in the intestinal form of lymphoma over the past 20 years and by the fact that several dietary modifications in cat food have occurred in a similar time frame in response to conditions such as urinary tract disease. Further investigation is warranted to prove or disprove such assertions.

Pathology and Natural Behavior

Lymphoma can be classified on the basis of anatomic location and histologic criteria. Several systems of anatomic classification exist for lymphoma in the cat. Some categorize the disease into mediastinal, alimentary, multicentric, nodal, leukemic, and individual extranodal forms. Others have combined various nodal and extranodal forms into categories of atypical, unclassified, and mixed, while still others have combined intestinal, splenic, hepatic, and mesenteric nodal forms into one category (intra-abdominal). For the purposes of this discussion, the "mixed" form is defined as cases involving multiple sites in which no primary site categorization was possible. Some discrepancies in the discussion of frequency inevitably will result from the variations in classification used in the literature. The relative frequency of anatomic forms and the immunophenotype may vary with geographic distribution and may be related to genetic and FeLV strain differences, as well as to the prevalence of FeLV vaccine use (see Table 31-6).

In most larger studies, most cases of lymphoma in cats (approximately 70% to 75%) are of the B-cell immunophenotype; however, the mediastinal, leukemic, and purely hepatic forms are more likely to be of T-cell derivation.[10,18] Cats with T cell–rich B-cell lymphoma (Hodgkin's-like lymphoma) and NK-like T-cell lymphoma (non-T, non-B-cell) have been described.[54,55] As with dogs, most lymphomas in cats have an intermediate-grade or a high-grade histology according to the working formulation (WF) criteria.[18,56]

Alimentary/intestinal lymphoma

Alimentary/intestinal lymphoma can manifest as a purely intestinal infiltration or as a combination of intestinal, mesenteric lymph nodes, and liver involvement.[11] Some reports limit the alimentary form to GI involvement, with or without extension to the liver. Approximately two thirds to three fourths of reported cases are of the B-cell immunophenotype. Most patients are older cats, and very few cases appear to be associated with FeLV antigenemia.[10,11,18,24-26,57-59] However, some discordance on these points is seen in the literature. In one smaller report, most alimentary cases sampled were found to be of the T-cell immunophenotype.[24] The population size was relatively small in comparison and may represent a geographic variation. The epitheliotropic form of intestinal lymphoma also has been reported to be more commonly T-cell in origin.[49,57] A Canadian group investigated formalin-fixed archival lymphoma tissue in cats and found a nearly even distribution between the B-cell and T-cell immunophenotype; they also found that approximately two thirds of the samples were FeLV positive by PCR techniques.[2,60-62] Some FeLV-negative tumors may be derived from transformation of multipotent lymphoid or monocyte precursors[61] or FIV-transformed B lymphocytes.[32,33,38,39]

The most common site of involvement in the alimentary tract is the small intestine (50% to 80% of cases), followed by the stomach (approximately 25%), ileocecocolic junction, and colon.[25,26,62] The tumor can be solitary or diffuse throughout the intestines (Figure 31-13), muscle layers, and intestinal submucosa, resulting in annular thickening that leads to partial or complete intestinal obstruction. In a series of colonic neoplasias

Figure 31-13

Necropsy specimen from a cat with intestinal lymphoma. Note the generalized thickening of the small intestine and the associated mesenteric lymph node involvement (arrows).

in cats, lymphoma was the second most common malignancy (41%), exceeded only by adenocarcinoma.[27] An abstract report has described chronic lymphocytic-plasmacytic enteritis in cats that progressed to overt lymphoma after 6 to 18 months of conservative therapy.[63]

Mediastinal form

The mediastinal form can involve the thymus and the mediastinal and sternal lymph nodes. Pleural effusion is common. In two large compilations, 63% of cats with thymic disease and 17% of cats with pleural effusion were documented as having lymphoma.[64,65] Occasionally the tumor extends from the thoracic inlet and can be palpated in the ventral neck region. Hypercalcemia is common with mediastinal lymphoma in dogs but is rare in cats with lymphoma.[66-68] PTHrP has been detected through immunoradiometric assays in cats with hypercalcemia of malignancy, including one cat with lymphoma.[69] Most cats with mediastinal lymphoma are young and FeLV positive, and their tumors are of the T-cell immunophenotype.*

Nodal forms

Involvement of peripheral lymph nodes alone is very unusual in cats with lymphoma, representing approximately 4% to 10% of cases.[10,11] In contrast, approximately one fourth of all other anatomic forms of lymphoma have some degree of lymph node involvement.[11] One third of cats with nodal lymphoma are of the T-cell immunophenotype and are FeLV antigenemic.[10,18] As lymphoma progresses, bone marrow infiltration with malignant cells and hepatosplenomegaly may develop.

Recently an uncommon and distinct form of nodal lymphoma in cats, referred to as *Hodgkin's-like lymphoma*, has been reported. This form typically involves solitary or regional nodes of the head and neck and histologically resembles Hodgkin's lymphoma in humans.[17,54,70] These tumors generally manifest as an enlargement of a single mandibular or cervical node and immunophenotypically are classified as T cell–rich B-cell lymphoma by immunohistochemical analysis.[54,70] None have been associated with either FeLV or FIV.

Several reports of nonneoplastic peripheral lymphadenopathy in cats have been published.[71-75] This condition resembles lymphoma clinically, and it has histologic features that also may resemble those of lymphoma.[71-75] Affected lymph nodes may be two or three times normal size. In one report, the syndrome was called *distinctive peripheral lymph node hyperplasia (DPLH)* of young cats.[72] These cats tend to be young (2 to 4 years old), and many have had episodes of fever or previous viral infections, or they may have hypergammaglobulinemia (polyclonal gammopathy); most are FeLV negative.[71] Histopathologically, the nodal architecture is severely distorted, showing loss of subcapsular and medullary sinuses. The cell population shows an admixture of histiocytes, lymphocytes, plasma cells, and immunoblasts and occasionally effaced lymphoid follicles. The lymph nodes regress spontaneously in most of these cats. The histologic changes noted in these lymph nodes resemble the histologic features of acquired immunodeficiency syndrome (AIDS)-related lymphadenopathy in humans.[72] In another report, benign lymphadenopathy in cats was associated with argyrophilic intracellular bacteria.[75]

Extranodal lymphoma

The most common extranodal sites for lymphoma are the kidneys, nasal cavity, eyes, retrobulbar space, CNS, and skin.

Renal lymphoma can be primary or associated with alimentary lymphoma. Based on several studies, the median age for cats with renal lymphoma is approximately 7.5 years. One fourth of cases are FeLV antigenemic, and most are of the B-cell immunophenotype.[10,11,18,76] The frequency of renal lymphoma is reported to be approximately 5% of all lymphomas. Extension to the CNS is a common sequela to renal lymphoma and occurs in 40% to 50% of treated cats.[76]

Nasal/paranasal lymphoma usually is a localized disease; however, systemic extension occasionally is seen.[10,77,78] Neoplasia accounts for most nonviral nasal/paranasal disease in cats, and lymphoma has been reported to represent nearly one third to one half of these cases.[79-81] It occurs primarily in older, FeLV-negative cats (median age, 9 to 12 years). At least three fourths of cases are B cell in origin, and most are intermediate grade or high grade histologically.[10,79-81] A subset of epitheliotropic T-cell nasal lymphomas has been reported.[81] Cats that are concurrently FeLV positive are more likely to have concurrent systemic disease.

In the older literature, CNS lymphoma was most often seen extradurally in the spinal canal of FeLV-positive cats (85% to 90%).[20,21,82] However, in more recent reviews, both spinal cord and intracranial lymphoma occurs mostly in older, FeLV-negative cats, and spinal cord sites are more commonly intradural than extradural.[83,84] After meningioma, lymphoma is the second most common tumor involving the CNS in cats.[84] Feline CNS lymphoma may be primary or may occur secondary to multicentric involvement (especially of the renal system or bone marrow).[83-86] Bone involvement is rarely seen radiographically.[87] Multiple cord regions and the brain are involved in nearly 50% of cats with spinal lymphoma, and more than 80% of these patients have other organ (e.g., renal) and bone marrow involvement.[20,21,83] Only one third of cases of intracranial

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*References 2, 10, 11, 17-20, 30, and 60.

lymphoma are primary and confined to the CNS, and all cats tested have been FIV negative.[84]

Cutaneous lymphoma generally is primary but can be seen secondary to multicentric involvement. It commonly is seen in older cats (median age, 10 to 12 years) and gender sex or breed predominance has not been found.[88-92] Although patients usually are FeLV negative, one case report using PCR techniques found evidence of FeLV provirus in tumor DNA.[92] Cutaneous lymphoma can be solitary or generalized. Two forms of cutaneous lymphoma have been distinguished histologically and immunohistochemically.[88-92] In most species, the epitheliotropic form, sometimes referred to as *mycosis fungoides*, is composed of T lymphocytes, whereas the nonepitheliotropic form usually is composed of B lymphocytes. In contrast, one report of nonepitheliotropic cutaneous lymphoma in cats found that five of six cases were of T-cell derivation.[93]

Neoplastic T lymphocytes are large and have abundant cytoplasm and convoluted nuclei (mycosis cells). They usually form intraepidermal nests of five to 10 cells, which are separated from surrounding keratinocytes by a clear space (Pautrier's microabscesses). The B-cell lymphomas show lymphocytes deep in the epidermis, with sparing of the papillary dermis and epidermis. A recent report described 23 cases of cutaneous lymphocytosis, an uncommon disease histologically resembling well-differentiated lymphoma.[94] Solitary lesions were most common, and all were composed primarily of T-cells, although two thirds had some B-cell aggregates. Cutaneous lymphocytosis was characterized as a slowly progressive disorder, but internal organ infiltration developed in a few cases.

A cat with cutaneous T-cell lymphoma and circulating atypical lymphocytes has been reported.[91] The circulating cells were lymphocytes with large, hyperchromatic, grooved nuclei. In humans, cutaneous T-cell lymphoma with circulating malignant cells is called *Sézary syndrome*,[95] which also has been reported in dogs.[96-99]

Histologic Grade Classification of Lymphomas

A number of histopathologic grading systems have been used to classify human NHL, including the WF (discussed earlier in the chapter). Most recently, the WF was used to classify more than 600 cases of feline lymphoma.[56] Low-grade lymphoma was found in 11% of the cases, intermediate-grade disease in 35%, and high-grade lymphoma in 54%. About 1.1% were plasmacytomas. More than one third of the tumors were the immunoblastic type. Lymphoblastic lymphoma, a subtype of the high-grade tumor, accounted for less than 3%. Similarly, in a large group of cases compiled in Australia (n = 118), 90% of the cases were medium- to high-grade disease as classified by the WF.[18]

Also as discussed earlier in the chapter, WHO has published a histologic classification scheme that uses the revised European American lymphoma (REAL) system as a basis for defining histologic categories of hematopoietic tumors of domestic animals.[100] This system incorporates both histologic criteria and immunohistologic criteria (B- and T-cell immunophenotype). The clinical relevance of this system is likely to be high; however, it awaits further investigation and evaluation.

In a series of 28 cases of alimentary lymphoma, 89% were the high-grade lymphoblastic type.[25] However, in another report, many of the intestinal lymphomas had several characteristics of MALT, as described in humans; that is, a tendency to remain localized in the lamina propria, a relatively indolent clinical behavior, and an excess of low-grade tumors.[56] The age of affected animals varied considerably with various subtypes, but in general, low-grade tumors tended to develop in older cats (over 10 years of age), and high-grade tumors tended to develop in younger cats (under 6 years of age).

A less commonly reported, distinct form of alimentary lymphoma has been described and classified as *large granular lymphoma*.[101-110] These lesions are granulated, round cell tumors that also have been called either *globule leukocyte tumors* or *large granular lymphocyte lymphoma*, although they probably are variations of the same disease. Large granular lymphocytes are characterized by abundant cytoplasm with prominent azurophilic granules. This population of cells includes NK cells and cytotoxic T cells. Several reports have identified their immunophenotype as CD3, CD57-like, perforin positive, and CD20 negative; these cells also have been described as having a T-cell receptor gene rearrangement. These tumors commonly originate in the small intestine, especially the jejunum or mesenteric lymph nodes; however, most cases show widespread metastasis to the lung, myocardium, salivary gland, and spinal cord. Leukemia also has been reported with this disease. Affected cats generally are FeLV negative. Large granular lymphocytes must be differentiated from several other granular cell types that may be found in the small intestine, including enterochromaffin cells, mast cells, and eosinophils.

History and Clinical Signs

The clinical signs associated with feline lymphoma vary and depend on the location and extent of disease.

The alimentary form is most commonly associated with an abdominal mass that originates in the GI tract. The condition often is associated with enlarged mesenteric lymph nodes or other organ involvement. In 50% to 85% of cases, a palpable abdominal mass or thickened bowel loops are present.[24,25,27,62,111] Clinical signs may consist of weight loss, anorexia, diarrhea, and

occasional vomiting. In approximately half of cases, the only historical finding is anorexia and weight loss.[25] Other reported presentations include abdominal distention, splenomegaly, persistent thrombocytopenia, and pica. Hematochezia and tenesmus may be present if the lymphoma involves the colon.[27] Polyuria and polydipsia have been reported in approximately 10% of cases.[62] In a small percentage of cases, the patient may have signs consistent with an acute abdomen as a result of intestinal perforation and concurrent peritonitis.[24]

Clinical signs of the mediastinal form of lymphoma include dyspnea, tachypnea, and a noncompressible anterior mediastinum with dull heart and lung sounds.[20,111-114] In rare cases, Horner's syndrome may be present as a result of involvement of the sympathetic nerve as it ascends around the first rib, and edema of the head may be caused by pressure on the cranial vena cava.[111] Pleural effusion is common; the pleural fluid is characterized by serohemorrhagic to chylous effusion, and in most cases neoplastic cells (lymphoblasts) are identified.[65,114]

Cats with the nodal form of lymphoma have variable clinical signs, depending on the location and extent of disease; however, these cats often are depressed and lethargic. Peripheral lymphadenopathy as the only physical finding is a very uncommon presentation for cats. As stated earlier, an uncommon and distinct form of nodal lymphoma in cats is Hodgkin's-like lymphoma. This form typically involves a single mandibular or cervical node, and the cat usually does not have any overt clinical signs.[54,70] In cats, unlike in dogs, peripheral lymphadenopathy without organomegaly is more often hyperplastic or reactive and does not represent lymphoma.[73]

The extranodal sites include the kidneys, skin, eyes, nasal area, and CNS. Renal lymphoma is most consistently bilateral, even in cats that appear to have unilateral disease.[76] In general, the kidneys are uniformly enlarged; however, they may also feel lumpy and irregular on palpation. More than 50% cats have signs consistent with renal insufficiency.[76]

Cats with CNS lymphoma most often have signs associated with thoracolumbar involvement.[22,23] The most common sites are between the second thoracic and fourth lumbar vertebrae. Signs include gradual or sudden onset of weakness, upper motor neuron paralysis to the bladder, tail flaccidity, hyperpathia in the region of the lesion, and progressing ataxia. The neurologic dysfunction may be insidious or may progress rapidly.[23] Cats with cervical spinal cord or nerve root involvement generally show peracute tetraparesis and diminished sensation in the thoracic limbs. Those with cervical root involvement may show root lesions (root signature), such as lameness and hyperesthesia upon shoulder extension. Common presenting signs for intracranial lymphoma, in decreasing order of frequency, include anorexia, ataxia, lethargy, altered consciousness, and aggression.[84]

Cats with nasal lymphoma usually have signs localized to the nasal passage. In a large compilation of cases, the presenting complaints, in decreasing order of frequency, were unilateral nasal discharge (bilateral is less common), facial deformity, dyspnea, and epistaxis.[79-81] Stertor, anorexia, epiphora, exophthalmia, sneezing, and regional lymphadenopathy also can occur.

Cutaneous lymphoma may be solitary or diffuse and manifest with alopecia, erythema, and crusted papules. Minimal peripheral lymphadenopathy may also be present. In most cats the signs have a prolonged duration (i.e., several months).[88-92,94]

Nonspecific signs

All cats with lymphoma, regardless of the site, may have secondary bone marrow infiltration that leads to anemia and a leukemic blood profile. Anemia is a common condition in cats with lymphoma, and at least 50% have moderate to severe nonregenerative anemia. Signs related to paraneoplastic hypercalcemia (PU/PD) can occur in cats, but the syndrome is much less common than in the dog. In one survey of hypercalcemia in cats, approximately 10% were diagnosed with lymphoma of various anatomic types.[115] One case of hypereosinophilic paraneoplastic syndrome and one case of symmetric cutaneous necrosis have been reported in cats with lymphoma.[116,117] A number of disease conditions can be confused with lymphoma in cats (Table 31-7).

Diagnostics and Clinical Staging

For most cats suspected of having lymphoma or leukemia, the diagnostic evaluation should include a CBC with differential cell count, platelet count, serum chemistry profile, and a test for FeLV and FIV. Bone marrow aspiration or biopsy may be indicated to evaluate for possible involvement and complete staging of the extent of disease. Bone marrow evaluation is particularly indicated if anemia, cellular atypia, and leukopenia are present. Histopathologic evaluation of lymph nodes or involved organ tissue (procured by means of surgical incision, endoscopy, or needle core biopsy) is essential for a definitive diagnosis. In the cat, lymph node fine-needle aspiration alone is not sufficient in most cases because of the difficulty involved in distinguishing lymphoma from benign hyperplastic lymph node syndromes unique to the species; these include idiopathic peripheral lymphadenopathy, plexiform vascularization of lymph nodes, and peripheral lymph node hyperplasia of young cats.[71-75] Whole lymph node excision is preferred in these cases, because determination of the orientation, invasiveness, and architectural

TABLE 31-7 Disorders That May Be Misdiagnosed as Feline Lymphoma

Form of Lymphoma	Possible Misdiagnosis
Thymic/mediastinal lymphoma	Thymoma, chylothorax, cardiomyopathy, pyothorax, feline infectious peritonitis (FIP), mesothelioma, diaphragmatic hernia
Alimentary lymphoma	Inflammatory bowel disease, FIP, intestinal carcinoma, foreign body, other intestinal tumors, hyperthyroidism
Peripheral lymph node lymphoma	Distinctive lymphadenopathy (reactive or hyperplastic), infection, feline immunodeficiency virus (FIV) infection
Renal lymphoma	Polycystic disease, FIP, acute renal failure, other renal tumors
Spinal/central nervous system lymphoma	Trauma, other tumors, FIP, discospondylitis, aortic embolism, IV disk herniation, mycotic infection, nonneoplastic feline leukemia virus (FeLV)–associated myelopathy
Nasal lymphoma	Rhinitis, inflammatory polyps, cryptococcosis, other tumors

abnormalities may be necessary for diagnosis. Additional site-specific cytologic or histologic assessments may be warranted when extranodal sites are suspected.

Histologic and cytologic samples also can be analyzed by various histochemical and immunohistochemical techniques to determine the immunophenotype (B cell or T cell), tumor proliferation rate (e.g., Ki-67, PCNA, AgNORs), and telomerase activity (see Chapter 14, section D), and histologic subtype (low, intermediate, or high grade).[10,18,26,34,43,44,46] The availability of such analysis is increasing, but currently none has proved to be predictive of outcome in cats.

Serum chemistry profiles can help establish the overall health and clinical staging of cats, but the results are not specific for a diagnosis. Elevated liver enzymes may indicate hepatic infiltration with lymphoma, and elevated blood urea nitrogen (BUN) and creatinine levels may indicate renal lymphoma. For cats with alimentary lymphoma, hypoproteinemia and anemia are reported to occur in up to 23% and 76% of cases, respectively.[25,118] Hypercalcemia is rare in cats but has been reported in patients with lymphoma at various anatomic sites.[25,66-68] Elevated globulin levels may indicate the presence of a monoclonal gammopathy with or without serum hyperviscosity (this is a rarely reported paraneoplastic syndrome in cats with lymphoma).[119,120] Hypoglycemia was reported in approximately one third of cats with lymphoma in an Australian study.[118] Serum alpha 1–acid glycoprotein concentrations have been reported to be elevated in cats with lymphoma, but no clinical relevance has been associated with this finding.[121]

Most cats with alimentary lymphoma develop a palpable abdominal mass or thickened intestinal loops. Approximately one third of patients have a mass that can be visualized on abdominal radiographs, and about 90% have ultrasound abnormalities.[24,25] The ultrasound abnormalities may include mesenteric lymphadenopathy in 33% to 50% of cases, an intestinal mass or thickening in approximately 40% of cases, and possibly splenomegaly (approximately 33% of cats with ultrasonographic changes in the spleen have lymphoma[122]), hepatomegaly, or effusion.[24,25] In one compilation of 28 cases, the lesions appeared ultrasonographically to be localized in 70% of cases and diffuse in 30%; however, at surgery only 33% were resectable.[25] For alimentary lymphoma, especially if primary GI lymphoma is suspected, caution must be exercised when endoscopically obtained tissue is used because of the difficulty in differentiating lymphoplasmacytic gastroenteritis from primary, diffuse, intestinal lymphoma.[123] However, diagnosis of this disease by means of endoscopically derived biopsies is improving with more advanced techniques and instrumentation. A wedge biopsy through serosa and muscularis, avoiding the mucosa, may be necessary to establish a diagnosis in cases in which endoscopic samples are equivocal. As an alternative, because nearly half of alimentary lymphomas have secondary mesenteric lymph node involvement, ultrasound-guided biopsy specimens or fine-needle aspirates may be adequate for a diagnosis.

For cats with mediastinal lymphoma, diagnostic suspicion may begin with a noncompressible cranial thorax on physical examination and conformation of a mediastinal mass or pleural effusion on a thoracic radiograph (Figure 31-14). Fine-needle aspiration of any suspected mass or cytologic evaluation of pleural fluid may be sufficient to establish a diagnosis. In most cats the finding of a monotonous population of lymphoblasts establishes a diagnosis.[65,124,125] However, definitive diagnosis of lymphoma in cats with a mediastinal mass and concurrent chylothorax can be challenging.[114] The CT appearance of these lesions has been

evaluated in only a small number of cats with mediastinal masses, and CT generally does not contribute to a definitive diagnosis.[126] If lymphoblasts are not identified in the pleural chylous effusion, cholesterol and triglyceride concentrations can be measured.[127] In chylous effusions, the pleural fluid triglyceride concentration is greater than in the serum; however, anorectic cats have lower triglyceride levels in the pleural fluid. A major differential diagnosis for mediastinal lymphoma is thymoma. The cytologic features of thymoma were recently described, and although these were found to be distinct from lymphoma in many cases, the diagnosis was challenging because of a preponderance of small lymphocytes in thymoma.[128] Mast cells can also be seen in up to 50% of aspirate samples from thymomas.

Diagnostics for most extranodal forms of lymphoma are site specific. In cats suspected of having spinal lymphoma, survey radiographs of the spine rarely reveal osseous lesions. Myelograms, CT, or MRI is indicated, and in approximately 75% of cases, an extradural or intradural mass is detected.[22,23,83,84] Most lesions occur at a thoracolumbar or lumbosacral location.[83] Fluoroscopic-guided, fine-needle aspiration of epidural lesions may yield enough tissue for a cytologic diagnosis. In most of the cats evaluated in one study, CSF analysis revealed a clear, colorless fluid with a mixed pleocytosis (mean, 140 cells/μl; range, 0 to 1625 cells/Ul) and an elevated protein content (mean, 140.7 mg/dl; range, 12 to 405 mg/dl).[23] Malignant lymphocytes were identified in six of 17 cats evaluated with CSF analysis. In a report of intracranial neoplasia, one of two cats with lymphoma had lymphoblasts in the CSF fluid.[84]

Bone marrow and renal involvement are common in cats suspected of having CNS lymphoma, and cytologic assessment of these organs generally is easier than in spinal sites. If nasal lymphoma is suspected, a biopsy specimen can be obtained either by intranasal procurement or by flushing one hemicavity with a bulb syringe and saline while occluding the contralateral cavity and collecting samples flushed out the nasopharynx (Figure 31-15). Thorough staging to make sure that the disease is confined to the nasal passages is recommended, because this presentation can be treated locally with radiotherapy if systemic involvement is ruled out. A CT scan is indicated to determine local involvement and to plan radiotherapy if it is to be pursued. For cutaneous lymphoma, punch biopsies (4 to 8 mm) should be taken from the most representative and infiltrative sites, although overtly infected skin lesions should be avoided.

Complete staging to rule out systemic disease is also recommended for cats with cutaneous lymphoma, because local therapies can be applied in cases of solitary disease. In the case of renal lymphoma, physical examination findings of massive and often bilateral renomegaly raise the index of suspicion. The radiographic appearance
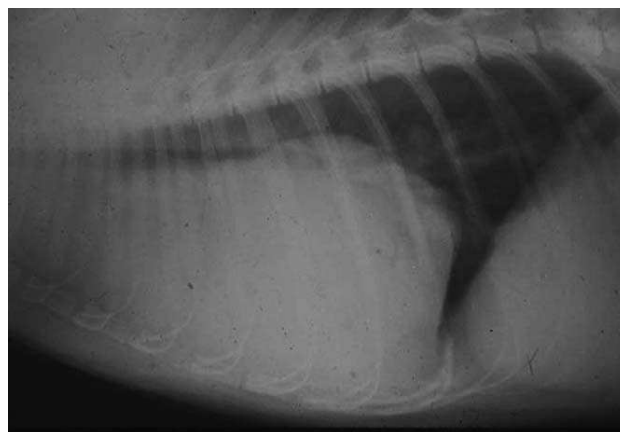
Figure 31-14

Left lateral thoracic radiograph of a young, feline leukemia virus (FeLV)-antigenemic cat with mediastinal lymphoma, which was confirmed on fine-needle aspirate. The cardiac silhouette is obscured by the large mass.
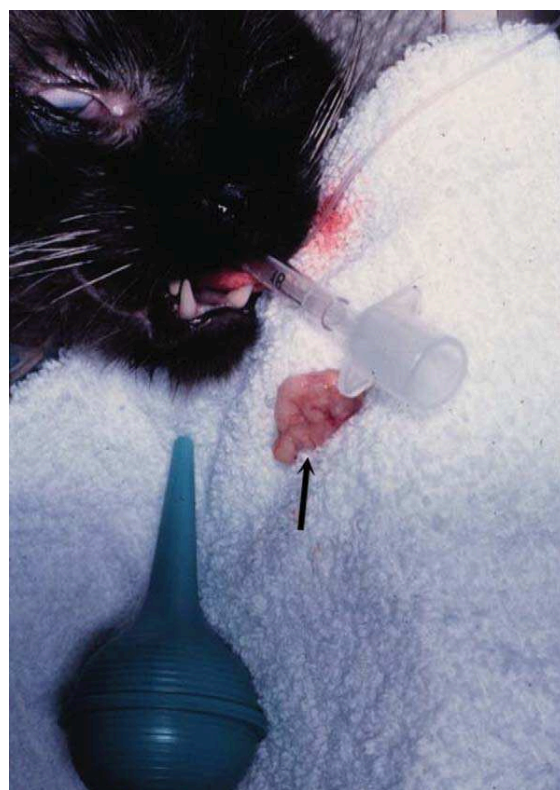
Figure 31-15

Flush biopsy of nasal lymphoma. Note the large sample *(arrow)* that was procured by retrograde flushing of saline through one nares while the contralateral nares was occluded. The sample is flushed through the pharynx and out the mouth.

A   B
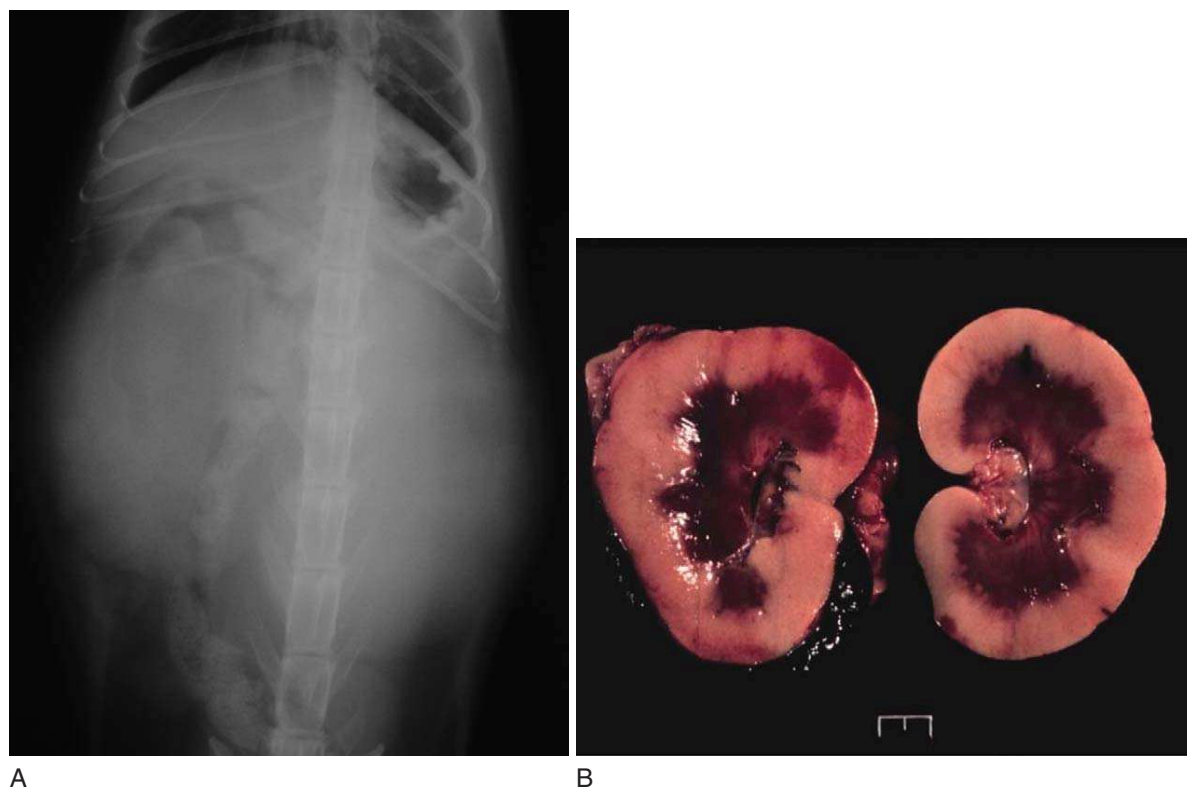
Figure 31-16

A, Ventrodorsal radiograph of a cat with renal lymphoma; note the massive, bilateral renomegaly.
B, Necropsy specimens from a cat with bilateral renal lymphoma show the diffuse cortical nature of the disease that is most common.

is a smooth to irregular renomegaly (Figure 31-16, *A*) that is most consistent with either renal lymphoma or polycystic kidney disease. The disease usually is diffuse throughout the renal cortex (Figure 31-16, *B*), and transabdominal needle aspiration or core biopsy is diagnostic in most cases.

A WHO staging system exists for the cat that is similar to the one used in the dog (see Box 31-1). However, because of the high incidence of visceral involvement in the feline species, another staging system is used more often (Box 31-5).[21] Because lymphoma in cats is more varied with respect to location and anatomic types, staging systems generally are less helpful for predicting response.

Advanced molecular diagnostics

Occasionally the diagnosis of lymphoma and the differentiation of malignant and benign proliferation of lymphocytes is not possible based on standard histologic and cytologic criteria. In these cases, advanced molecular analyses may be helpful for confirming a diagnosis. Clonality is the hallmark of malignancy; that

is, the malignant cell population theoretically is derived from expansion of a single malignant clone. This was discussed earlier in the chapter for lymphoma in the dog (see Section A and Figure 31-8), and the same assays can be applied for advanced diagnosis of lymphoma in cats.[85] Investigators have used PCR techniques to amplify the variable regions of the T-cell and immunoglobulin receptor genes to detect clonal lymphocyte populations in cats, and these techniques appear to be a useful adjunctive diagnostic tool, although they are undergoing a more thorough assessment. Molecular techniques may also prove useful for assessing early recurrence, for more accurate clinical staging, and for providing so-called molecular remission rates, because they are more sensitive than the standard cytologic assessment of peripheral blood, bone marrow, or lymph nodes.

Treatment of Malignant Lymphoma

Our knowledge base for treating cats with lymphoma is less well established and less predictable than that for

Clinical Staging System for Feline Lymphoma

Stage 1
- Single tumor (extranodal) or single anatomic area (nodal)
- Includes primary intrathoracic tumors

Stage 2
- Single tumor (extranodal) with regional lymph node involvement
- Two or more nodal areas on the same side of the diaphragm
- Two single (extranodal) tumors with or without regional lymph node involvement on the same side of the diaphragm
- Resectable primary gastrointestinal tract tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only

Stage 3
- Two single tumors (extranodal) on opposite sides of the diaphragm

- Two or more nodal areas above and below the diaphragm
- All extensive, primary, unresectable, intra-abdominal disease
- All paraspinal or epidural tumors, regardless of other tumor site or sites

Stage 4
- Stage 1, 2, or 3 tumor or tumors with liver or spleen involvement (or both)

Stage 5
- Stage 1, 2, 3, or 4 tumor or tumors with initial involvement of the central nervous system or bone marrow (or both)

Modified from Yunis JJ, Oken MM, Theologides A et al: Recurrent chromosomal defects found in most patients with non-Hodgkin's lymphoma, Cancer Genet Cytogenet 13:17-28, 1984.

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dogs, primarily because of the greater variation in histologic type and anatomic locations observed in felines. The chemotherapeutic agents most often used to treat feline lymphoma are similar to those used for dogs and humans with lymphoma (see Section A); they include doxorubicin, vincristine, cyclophosphamide, methotrexate, L-asparaginase, CCNU, and prednisone.* In general, cats tolerate chemotherapy quite well. Selected published protocols for the treatment of feline lymphoma are detailed in Table 31-8.

Most current combination protocols in North America are modifications of CHOP protocols initially designed for human oncologic use. In Europe, COP (i.e., without the addition of doxorubicin) is used more often in cats with lymphoma, and one compilation reported results similar to those for CHOP.[133] Some studies with relatively few case entries have reported limited activity for doxorubicin as a single agent in cats with lymphoma[19,132]; however, larger studies using combination protocols have more consistently reported that the addition of doxorubicin is necessary to attain more durable responses.[10,129] In European and Australian studies that reported less favorable doxorubicin responses, the patient populations consisted of a higher proportion of the mediastinal form in Siamese cats, a population less frequently included in North American reports.[19,133]

The protocol the authors use in their practice is presented in Table 31-9. This protocol has been used in many cats with various forms of lymphoma and is well tolerated. Currently, our canine lymphoma protocols (see Section A) discontinue chemotherapy by week 25, and we have sufficient data exist to show that in dogs, this short, maintenance-free protocol is as good as if not superior to longer maintenance protocols. Because long-term maintenance protocols have not proved superior to maintenance-free protocols in dogs and humans with lymphoma, the same likely holds true in the cat, although no data exist to document this. Until such time as evidence to the contrary exists, we recommend discontinuation of chemotherapy at week 25 in cats that have attained complete remission.

Although doxorubicin appears to be the most effective agent for treating cats with lymphoma in North America, the species generally is less tolerant of doxorubicin than are dogs, and significant toxicity results when it is used at the dog dosage of 30 mg/m^2 given intravenously every 3 weeks. However, at lower dosages (e.g., 25 mg/m^2 or 1 mg/kg, given intravenously) doxorubicin can be used without significant toxicity.[10,129] The major toxicities noted with doxorubicin are anorexia, myelosuppression, renal toxicity and, if perivascular leakage occurs, severe tissue damage. Clinical doxorubicin-induced cardiac toxicity has not been documented in cats, but no information indicates that cats are resistant to myocardial damage. Renal toxicity has been produced experimentally in rats and

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*References 1, 5, 6, 10, 19, 24, 26, 76, and 129-135.

TABLE 31-8 Selected Treatment Protocols for Feline Lymphoma*

Protocol	Tumor Type	Cases	Complete Remission (%)	Median Remission† (mo)	Median Survival (mo)	References
University of Wisconsin (UW) long-term maintenance protocol						
Vincristine, IV, weeks 1, 3, 6, 8, 11, 12, 15, 19, and 23	A. All types	22	68	9.1	7.5	10
L-asparaginase, 400 IU/kg, IM, week 1	B. All types	38	47	CR: 21.8 PR: 3.8	CR: 21.8 PR: 4	187
Cyclophosphamide, 250 mg/m², IV, weeks 2, 7, 13, and 21						
Doxorubicin, 20 mg/m², IV, weeks 4, 9, and 25						
Methotrexate, 0.8 mg/kg, IV, week 17						
Prednisone, 2 mg/kg, PO, daily for 2 weeks, then continuous at 1 mg/kg daily						
The protocol is continued in sequence biweekly as described for weeks 11 through 25 for 12 months, then triweekly for 6 months, then monthly for 6 months.						
COP						
A. Cyclophosphamide, 300 mg/m², PO, every 3 weeks	A. All types	NR	79	5	NR	1
Vincristine, 0.75 mg/m², IV, every 3 weeks	Thymic	12	92	6	NR	
Prednisone, 2 mg/kg, PO, continuously	Alimentary	7	86	4.5	NR	
Treatment is continued for 1 year.	Peripheral nodes	5	80	2.8	NR	
	Multicentric	4	100	5	NR	
B. As in A, *except* vincristine is given weekly for 4 weeks, then every 3 weeks.	B. All types	61	75.4	8.4	8.9	133
	Mediastinal	22	81.8	8.4	8.9	
	Alimentary	11	63.6	8.2	6.4	
	Peripheral	7	85.7	12.6	20.8	
	Nasal	8	75	11.9	11.9	
	Alimentary	28	33	7	1.5	26
	All types	38	47	3‡	NR	129
	All types	52	52	3	3/2	10
VCM						
Vincristine, 0.025 mg/kg, IV, week 1	Overall	NR	52	NR	2	6
Cyclophosphamide, 10 mg/kg, IV, week 2	Thymic	31	45	2	2.6	
Vincristine, 0.025 mg/kg, IV, week 3	Alimentary	9	50	6	9.6	
Methotrexate, 0.8 mg/kg, IV, week 4	Renal	6	16	NR	5	
Repeat weekly treatments as above.	Multicentric	16	68	NR	18	
Continue prednisone at 5 mg, PO.						

Mediastinal involvement: L-asparaginase (400 mg/kg) is given at the first treatment.
Treatment is continued for 2 years.

Modified VCM: L-asparaginase
Same protocol as for VCM *except* prednisone (2 mg/kg, PO) is given continuously

L-asparaginase, 400 IU/kg, IM, week 1 with vincristine

Renal lymphoma only: After CR, substitute cytosine arabinoside, 600 mg/m², SQ, divided into four doses over 2 days in place of cyclophosphamide.

Treatment is continued for 2 years.

COP + doxorubicin
COP is given per UW protocol A. Once CR is achieved, patient is switched to doxorubicin, 25 mg/m², IV, every 3 weeks for 6 months.

AMC
Vincristine, 0.025 mg/kg, IV, weeks 1, 4, 8, and 12
L-asparaginase, 400 IU/kg, IM, week 1
Cyclophosphamide, 10 mg/kg, IV, weeks 2, 5, and 10
Doxorubicin, 20 mg/m², IV, week 3,
Methotrexate, 0.8 mg/kg, IV, week 14
Repeat weekly treatments as above.
Prednisone, 5 mg, PO, twice daily, continuously
Protocol is continued in sequence biweekly as described for weeks 8 through 14 for 12 months, then triweekly for 6 months, then monthly for 6 months.

Doxorubicin alone
A. 25 mg/m² or 1 mg/kg every 3 weeks for five treatments
B. As in A, but with only three treatments

Protocol	Type				
Modified VCM: L-asparaginase (Renal)	Renal	28	61	5	5.7	77
Renal lymphoma only	All types	103	62	7	7	5
COP + doxorubicin	All types	7	47	9.4	NR	129
AMC	Alimentary	31	71	PR/CR: 4	Overall: 6.7 CR: 8.6	27
Doxorubicin alone A	A. All types	21	26	3	NR	132
Doxorubicin alone B	B. All types	21	32	NR	NR	19

IV, Intravenous; IM, intramuscular; PO, oral; CR, complete remission; PR, partial remission; NR, not reported.

*Few of these studies involved a large number of cats, and fewer compared protocols in a randomized, prospective fashion. Also, staging, inclusion, and response criteria varied considerably among the protocols. For these reasons, evaluation of the efficacy of the various protocols is subject to bias, therefore the protocols should be compared with caution.

†Reported remission times often are longer than survival times because only cases in which remission is achieved are included in the remission time, whereas both responders and nonresponders are included in the survival calculation.

‡For 11 cats that received only COP.

TABLE 31-9	CHOP-Based Chemotherapy Protocol for Cats with Lymphoma*
Treatment Week	**Drug, Dosage, and Route**
1	Vincristine, 0.5-0.7 mg/m², IV
	L-asparaginase, 400 U/kg, SC
	Prednisone, 2 mg/kg, PO
2	Cyclophosphamide, 200 mg/m², IV
	Prednisone, 2 mg/kg, PO
3	Vincristine, 0.5-0.7 mg/m², IV
	Prednisone, 1 mg/kg, PO
4	Doxorubicin, 25 mg/m², IV
	Prednisone, 1 mg/kg, PO[†]
6	Vincristine, 0.5-0.7 mg/m², IV
7[‡]	Cyclophosphamide, 200 mg/m², IV
8	Vincristine, 0.5-0.7 mg/m², IV
9	Doxorubicin, 25 mg/m², IV
11	Vincristine, 0.5-0.7 mg/m², IV
13[‡]	Cyclophosphamide, 200 mg/m², IV
15	Vincristine, 0.5-0.7 mg/m², IV
17	Doxorubicin, 25 mg/m², IV
19	Vincristine, 0.5-0.7 mg/m², IV
21[‡]	Cyclophosphamide, 200 mg/m², IV
23	Vincristine, 0.5-0.7 mg/m², IV
25[¶]	Doxorubicin, 25 mg/m², IV

*A complete blood count (CBC) should be performed before each chemotherapy treatment. If the neutrophil count is below 1500 cells/Ul, the practitioner should wait 5 to 7 days, repeat the CBC, and then administer the drug if the neutrophil count has risen above the 1500 cell/UL cutoff.

[†]Prednisone (1 mg/kg, PO) is continued every other day from this point on.

[‡]If renal lymphoma or central nervous system (CNS) lymphoma is present, substitute cytosine arabinoside (600 mg/m², divided, SC twice daily) over 2 days for these treatments.

[¶]If the patient is in complete remission at week 25, therapy is discontinued and the cat is rechecked monthly for recurrence.

IV, Intravenous; SC, subcutaneous, PO, oral.

rabbits and has been reported in cats.[136-138] In our experience, the incidence is significant enough that renal function in cats should be monitored closely (i.e., serial creatinine and urine specific gravity analysis) before each therapy session. A stealth liposomal form of doxorubicin has been shown to increase the likelihood of renal toxicity and therefore is limited in its use in this species.[138] A small number of cats with lymphoma have been treated with single agent CCNU at dosages ranging from 30 to 60 mg/m² given orally every 3 to 6 weeks.[130,131]

Although activity was noted, only partial responses were reported.

Little is known about the treatment of solitary or regional nodes of the head and neck that closely resemble Hodgkin's lymphoma histologically.[1,54] One study described the clinical outcome in four cats with this condition; these researchers found that the course of disease was prolonged, but after surgical excision of the single affected node, only one cat showed recurrence 6 months after surgery.[54] A second surgery was performed in this cat, and a second recurrence was noted, again 6 months after excision.

Cats with granular cell lymphoma or globule leukocyte tumors tend to respond poorly to chemotherapy, although durable responses have been reported.[102] Currently, too few cases have been treated with aggressive chemotherapy to allow an assessment of response to therapy for this disease.

A distinct form of intestinal lymphoma in cats that is composed of small, mature lymphocytes has been referred to as *lymphocytic lymphoma*. This syndrome has been reported to respond well to oral prednisone (10 mg/cat/day) and chlorambucil (15 mg/m² given once daily for 4 consecutive days out of every 3 weeks). A complete response rate of 69% was reported, with median remission and survival durations of 16 and 17 months, respectively.[62]

Radiation therapy has been used effectively to treat localized lymphoma, such as epidural, mediastinal, and nasal lymphoma. Total doses of 10 to 15 Gy usually result in a complete remission.[77] In one study, 10 cats with localized lymphoma were treated with radiation, alone or with chemotherapy, at doses of 8 to 40 Gy.[78] Eight of the 10 cats achieved complete remission, with a median remission duration of just over 2 years.

Radiation therapy also has been used effectively to treat nasal lymphoma.[78] Complete response is the norm (80% to 100%), and median remission durations exceeding 1.5 years can be expected in FeLV-negative cats with disease confined to the nasal and paranasal cavities. For nasal lymphoma, radiotherapy appears to be superior to chemotherapy, for which reported median remission durations are 151 to 380 days.[10]

Very little has been published about the treatment of cutaneous lymphoma or mycosis fungoides in cats; however, a report of a complete response to CCNU exists.[135] Cats with a solitary mass should be treated with surgical excision, although clinical staging is necessary to rule out possible internal involvement. Combination chemotherapy can be considered if multiple sites are involved. If the disease is localized to a small region, radiation therapy usually is also effective. Mycosis fungoides may be treated effectively in dogs with retinoids, such as isotretinoin (Accutane) at a dosage of 3 to 4 mg/kg given orally daily or etretinate (Tegison) at a dosage of 1.25 mg/kg given orally daily; however, no

clinical studies have been published demonstrating efficacy in cats.

General Treatment Recommendations

Providing precise treatment recommendations for the wide variety of clinical settings of cats with lymphoma is difficult. Our current recommendation is to treat cats with lymphoma using the CHOP- based protocols (see Table 31-9). Doxorubicin alone (25 mg/m^2 given IV every 3 weeks for five total treatments) or palliative prednisone therapy is offered if the client declines more aggressive therapy.

Nutritional support is especially important, particularly for cats with alimentary lymphoma. A feeding tube may need to be placed in cats undergoing chemotherapy for alimentary lymphoma, particularly if anorexia is a factor (see Chapter 16, section B.)

Ultimately, most cats with lymphoma that are successfully treated with chemotherapy have a relapse of the disease. This often represents a recrudescence of the tumor in a more drug-resistant form. At the first recurrence, reinduction first should be attempted by using the induction protocol that was successful initially. In general, the likelihood of a response and the length of the response are half those for the initial therapy; however, a subset of animals enjoy long-term reinduction.

If reinduction fails or if the cat does not respond to the initial induction, so-called rescue agents or rescue protocols can be attempted. These are drugs or drug combinations that typically are not found in the standard CHOP protocol and are withheld for use in drug-resistant cases. A number of rescue protocols have been reported in the veterinary literature and were reviewed previously.[139,140] The most common rescue protocols advocated for cats with resistant relapse include single agent use or combination use of mitoxantrone, doxorubicin (if doxorubicin was not part of the original induction protocol), CCNU, and MOPP. In general, overall rescue response rates of 40% to 50% are reported, but these responses usually are not durable; median responses of 1.5 to 2 months are the norm. A small subset of animals enjoy longer rescue durations.

Prognosis

In general, cats do not enjoy response rates as high or remission and survival durations as long as dogs with lymphoma. Complete response rates range from 50% to 70% after combination chemotherapy, and overall median remission and survival durations are approximately 4 and 6 months, respectively.[1,5,6,10,129] However, a significant proportion of cats (25% to 30%) that achieve a complete response with combination chemotherapy enjoy more durable overall remission and survival times (i.e., 1 year or longer).

The response rate and the length of response vary according to the presence or absence of several prognostic factors. The wide variation in frequency and the great heterogeneity of anatomic forms of lymphoma in cats make specific prognostications more difficult than in dogs with lymphoma. Most studies have lumped anatomic groups together to produce remission and survival data, and individual group numbers generally have been too small to apply statistical analysis with meaningful power. That being said, the factors that appear to be most strongly associated with a more positive prognosis in cats are a complete response to therapy (Figure 31-17), which unfortunately cannot be determined before treatment; negative FeLV status (Figure 31-18); early clinical stage (Figure 31-19); anatomic location; and addition of doxorubicin to the treatment protocol.[5,10,24,129] Unlike in the dog, CD3 immunoreactivity has not been established as a negative prognostic factor in the cat.[10] Early reports may contradict more recent studies partly because of the decline in FeLV-associated lymphoma, and patients reported in the early literature may not equate to more recent populations studied. In general, FeLV-negative cats that achieve a complete response on CHOP-based protocols have a high likelihood of long-term survival,

CR (MS=253d; N=54)
PR (MS=48d; N=10)
PD (MS=46d; N=26)
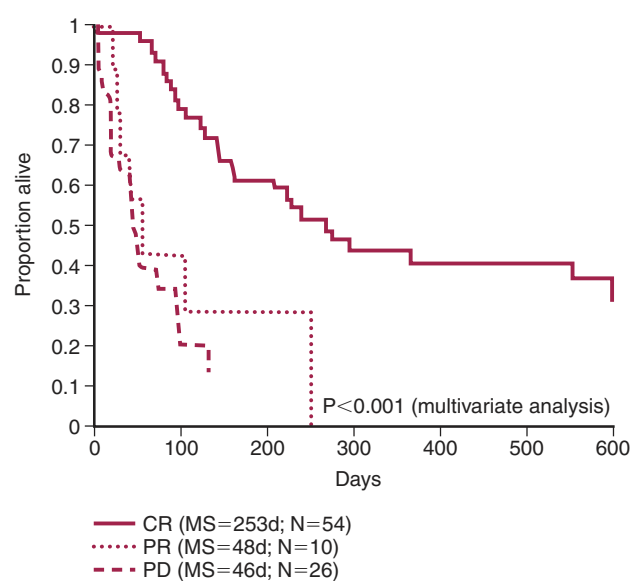
Figure 31-17

Kaplan-Meier curve showing survival time estimates stratified by response to therapy. Cats in which a complete response *(CR)* was achieved had a significant survival advantage compared to cats in which only a partial response *(PR)* was achieved or that showed progressive disease *(PD)*. Note the long tail in the CR group, representing the approximately 35% of patients that had durable remissions. *(From Vail DM et al: J Vet Intern Med 12: 349-354, 1998.)*
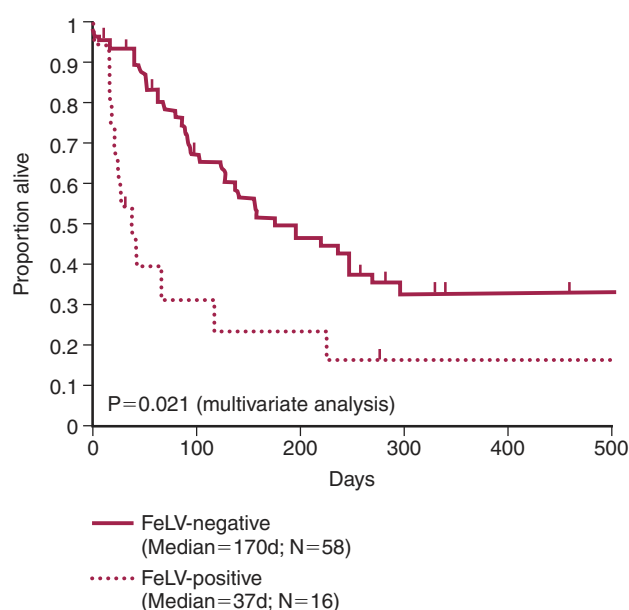
Figure 31-18

Kaplan-Meier curve showing survival time estimates stratified by feline leukemia virus (FeLV) results on an enzyme-linked immunosorbent assay (ELISA). FeLV-negative cats had a significant survival advantage compared to FeLV-antigenemic cats. *(From Vail DM et al: J Vet Intern Med 12: 349-354, 1998.)*

Figure 31-19

Kaplan-Meier survival curve showing the correlation between clinical stage of disease and survival after combination chemotherapy with vincristine/cyclophosphamide/methotrexate (VCM)–L-asparaginase. Cats with stage I or II disease had a significant survival advantage compared to more those with advanced disease (stages III through V). *(From Mooney SC et al: J Am Vet Med Assoc 194:696-702, 1989.)*

with approximately 30% alive at 1.5 years after diagnosis.

Location-specific prognosis

For cats with alimentary lymphoma, median survival times of 7 to 10 months are expected with chemotherapy that includes doxorubicin.[10,26,129] In one study of 28 cats with alimentary lymphoma in which the treatment protocol did not include doxorubicin, the median survival time was only 50 days.[25]

Mediastinal lymphoma in FeLV-positive young cats is associated with the poorest prognosis, and survivals times of approximately 2 to 3 months are expected with chemotherapy.[6,10,19] In contrast, older, FeLV-negative Siamese cats with mediastinal lymphoma appear to experience remission rates approaching 90%, and responses tend to be quite durable.

Overall, cats with nasal lymphoma have the best prognosis, because local radiotherapy (or chemotherapy if radiotherapy is not available) generally results in excellent control, with median survival times approaching 1.5 years.[10,78] However, cats with nasal lymphoma and concurrent FeLV infection have much shorter survival durations.

Renal lymphoma is associated with a shorter survival time, with published medians ranging from 3 to 6 months.[1,6,10,76] In a study by Mooney and colleagues,[76] 28 cats with primary renal lymphoma were treated with combination chemotherapy. A complete response (CR) was noted in 17 cats (61%), and nine (32%) had a partial response. The median survival times were 4 months for the cats that showed a complete response and 1 month for those that showed a partial response. The duration of response to chemotherapy did not correlate with the degree of renal insufficiency except in cats with a BUN higher than 150 mg/dl. Extension to the CNS occurred in 40% of these treated cats. The investigators revised their chemotherapy protocol to include cytosine arabinoside, which can penetrate the blood barrier, theoretically to prevent or reduce CNS metastasis; however, definitive improvement with this modification has not been documented.

Few studies have reported on the results of treatment of cats with spinal lymphoma. In one study of four cats treated with chemotherapy (L-asparaginase, vincristine, and prednisone) combined with spinal radiation (n = 3) or surgical cytoreduction (n = 1), most of the cats were euthanized by 5 months, although one cat survived 13 months.[23] In another study of nine cats treated with chemotherapy (vincristine, cyclophosphamide, and

prednisone), three cats achieved a complete response with a duration of 14 weeks and three achieved a partial response with a duration of 6 weeks.[22] One cat treated with dorsal decompression laminectomy and chemotherapy survived 13 months. Although the numbers are small overall, the prognosis for spinal lymphoma is poor.

FELINE LEUKEMIAS, MYELOPROLIFERATIVE DISORDERS, AND MYELODYSPLASIA

Section C of this chapter presents a complete discussion of leukemias and MPDs, including a general discussion of hematopoiesis, etiologies, lineage classification, and descriptions.

The classification of leukemias in cats is difficult because of the similarity of clinical and pathologic features and the transition, overlap, or mixture of cell types involved.[110,141-148]

Leukemia is a neoplastic proliferation of hematopoietic cells that originate in the bone marrow. Cell lineage includes myeloid cells, neutrophils, basophils, eosinophils, monocytes, lymphoid cells, megakaryocytes, and erythrocytes. Box 31-6 presents a classification scheme for the leukemias identified in the cat. Leukemias also must be classified according to their degree of differentiation.[110] Well-differentiated leukemia is referred to as *chronic* leukemia, and poorly differentiated leukemia usually is called *acute* leukemia; this distinction is very important in the therapeutic management and prognosis of leukemias. Cats with *acute leukemia* usually show signs of severe anemia (pale mucous membranes), splenomegaly, and febrile episodes. Cats with chronic leukemia may have a longer duration of signs and mild anemia with or without splenomegaly.

In cats suspected of having leukemia, bone marrow aspiration or biopsy usually is diagnostic. The preferred sites for bone marrow aspiration are the proximal humerus and the iliac crest. Cats with acute leukemia are likely to have malignant cellular infiltrates in organs other than the bone marrow.[144] The bone marrow aspirate must contain more than 30% abnormal blast cells to support a diagnosis of an acute leukemia. In cats suspected of having CLL, infiltration of the bone marrow with more than 20% mature lymphocytes helps confirm the diagnosis. All cats with leukemia should be tested for FeLV and FIV. Determining the lineage of some leukemias can be challenging; most can be distinguished from one another by histologic appearance, histochemical stains, immunohistochemical analysis of cell surface antigens, and flow cytometric analysis of the leukemic cells for cellular antigens that identify their lineage.[141,143,149,150] In addition, examination of blast cells by electron microscopy

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Box 31-6	**Classification of Leukemia in Cats**

Myeloproliferative disorders
Granulocytes
Granulocytic (myeloid, neutrophilic) leukemia
Eosinophilic leukemia
Basophilic leukemia

Monocytes
Monocytic leukemia

Erythrocytes
Erythremic myelosis
Primary erythrocytosis

Megakaryocytes
Megakaryocytic leukemia (myelosis)
Primary thrombocythemia

Miscellaneous
Mast cell leukemia
Myelofibrosis
Malignant histiocytosis
Undifferentiated leukemia

Mixed cell lines
Myelomonocytic leukemia (neutrophils and
   monocytes)
Erythroleukemia (erythrocytes and granulocytes)
Myelodysplastic syndrome

Lymphoproliferative disorders
Lymphocytes
Acute lymphoblastic leukemia
Chronic lymphocytic leukemia

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may reveal characteristic ultrastructural features. The French-American-British (FAB) classification system (Box 31-7) is considered useful in cats with myelodysplastic syndromes, and almost all these patients are FeLV antigenemic.[151,152]

Lymphoid Leukemia

Lymphoid leukemia is the most common leukemia in cats. Approximately 25% of cats with lymphoma also have a leukemic blood profile.[153] ALL is the most common lymphoid leukemia.[154] ALL is characterized by the presence of poorly differentiated lymphoblasts and prolymphocytes in the blood and bone marrow.[155] Most cats with ALL have normal to low WBC counts. A few cats have leucocytosis with circulating blasts. A moderate to marked anemia is common. Bone marrow aspiration usually reveals extensive infiltration with lymphoblasts. Approximately 60% to 80% of cats

	French-American-British (FAB) Classification System for Subtypes of Leukemias and Dysplasias
Box 31-7	

Acute leukemias

AUL   Acute undifferentiated leukemia (formerly called reticuloendotheliosis)
M1   Myeloblastic leukemia without differentiation
M2   Myeloblastic leukemia with some neutrophilic differentiation
M3   Promyelocytic leukemia (not recognized in animals)
M4   Myelomonocytic leukemia
M5a   Monocytic leukemia without differentiation
M5b   Monocytic leukemia with some monocytic differentiation
M6   Erythroleukemia
M6Er Variant of M6 with erythroblasts comprising erythroid component
M7   Megakaryoblastic leukemia

Chronic myeloid leukemias

CML   Chronic myelogenous leukemia
CMML Chronic myelomonocytic leukemia
CMoL Chronic monocytic leukemia

Hematopoietic dysplasia

MDS   Myelodysplastic syndrome
MDS-Er Myelodysplastic syndrome with erythroid predominance

with ALL are FeLV positive, and most malignant cells have a T-cell phenotype.[156]

CLL, which is rarely reported in cats, is characterized by the presence of well-differentiated, small, mature lymphocytes in the peripheral blood and bone marrow.[155,156] Although most of these cells are of the T-cell lineage (particularly T helper), B-cell CLL has been reported.[150,157,158] Most cats with CLL have an elevated WBC count (i.e., over 50,000/μl), and most are FeLV negative.

Granulocytic Leukemia (Myeloid, Neutrophilic)[141-144]

The total leukocyte count varies and may range from leukopenia to marked leukocytosis. Chronic granulocytic leukemia (GL) must be distinguished from leukemoid reactions associated with infections. Acute GL is characterized by a large percentage of myeloblasts and/or progranulocytes in the blood and bone marrow. Myeloblasts can be difficult to distinguish from lymphoblasts, but the former have a finer chromatin pattern, a smaller nucleus-to-cytoplasm ratio, more prominent or multiple nucleoli, and sometimes cytoplasmic granules. It is not uncommon in cats with GL to have no recognizable neoplastic cells in the peripheral blood. The bone marrow is hypercellular as a result of granulocytic leukemia cells.

Myelomonocytic Leukemia[141,145,146,159]

Myelomonocytic leukemia (MML) results from malignant transformation of both neutrophils and monocytes. This form of leukemia is one of the most common forms reported.

Monocytic Leukemia[143,146,147]

Monocytic leukemia (ML) is a rarely reported leukemia. It generally is considered an acute leukemia regardless of the morphologic appearance of the cells.

Eosinophilic Leukemia

Eosinophilic leukemia (EL) is rarely diagnosed in cats and is considered a variant of chronic granulocytic or myeloid leukemia.[148,160] FeLV has induced EL experimentally.[160] Mature eosinophils outnumber immature stages, and anemia is uncommon in cats associated with EL. Cats usually have an eosinophil count of 15,000 cells/μl or higher with or without immature cells in the peripheral blood. The bone marrow shows hyperplasia of eosinophilic precursors, and the myeloid to erythroid ratio (M:E) is significantly increased. Organ infiltration, such as in lymph nodes, the spleen, and the liver, can be seen.[161] In establishing a diagnosis of EL, it is important to rule out eosinophilic enteritis, parasitism, eosinophilic granuloma complex, and allergic disorders. Diagnosing EL can be very difficult because of the hypereosinophilic syndrome (HES) seen with other disease conditions in cats.[161-163] HES is characterized by a marked increase in the eosinophil count, bone marrow hyperplasia of eosinophilic precursors, and multiple organ infiltration by mature eosinophils. Most cats have signs related to GI involvement.

Basophilic Leukemia[164]

Basophilic leukemia (BL) is considered a variant of chronic granulocytic leukemia. Only one confirmed case of basophilic leukemia has been reported in the cat. It is important to differentiate BL from systemic mastocytosis with mast cell leukemia. Mast cells have numerous cytoplasmic granules and round nuclei. Basophils have segmented nuclei and cytoplasmic granules that superimpose on the nucleus, giving it a moth-eaten appearance.

Erythremic Myelosis[165-168]

Erythremic myelosis (EM) is an MPD characterized by excessive proliferation of erythroid elements, which results in an increase in nucleated erythrocytes (rubriblasts to metarubricytes). Severe anemia is common, and the peripheral blood shows numerous nucleated erythrocytes, moderate to marked anisocytosis, and an increased erythrocyte mean cell volume. The bone marrow contains a preponderance of normal-appearing erythrocyte precursors. Some cats undergo blast transformation to myeloblastic, granulocytic, or a poorly differentiated leukemia (previously called reticuloendotheliosis). The transition from erythremic myelosis to erythroleukemia to acute granulocytic leukemia is well recognized in humans.[169]

Erythroleukemia (Reticuloendotheliosis)[110,143,146]

Erythroleukemia, or acute erythremic myelosis, can develop from blast transformation of erythremic myelosis. Primitive erythroid precursors in the blood and bone marrow predominant. Primitive cells resembling myeloblasts often are present in low numbers.

Primary Erythrocytosis[170-173]

Primary erythrocytosis is rarely reported in cats, and the diagnosis is based on an elevated PCV (65% to 80%) with low to normal serum erythropoietin activity. Most clinical signs are associated with an increased red blood cell (RBC) volume, which increases blood volume and viscosity, causing impaired blood flow, stasis, and tissue hypoxia. Neurologic signs such as seizures, blindness, and mental depression are common.[172] The oral mucous membranes may appear brick red. It is important to differentiate this condition from secondary polycythemia caused by renal tumors, chronic hypoxia, and right-to-left cardiac shunts.

Megakaryocytic Leukemia[174-176]

Megakaryocytic leukemia is characterized by abnormal megakaryocytic hyperplasia in the bone marrow. The megakaryocytes are morphologically abnormal, and some are small (dwarf megakaryocytes) and have few or no nuclear lobulations. Thrombocytopenia or thrombocytosis may be present. In humans this form of leukemia often is associated with extensive marrow fibrosis and an increase in reticulum or collagen.[169]

Primary Thrombocythemia[177]

Primary thrombocythemia is a very rare, chronic MPD characterized by proliferation of megakaryocytes and elevated platelet counts exceeding 1 million. Giant platelets and platelets with abnormal morphology may be seen in the peripheral blood. One case has been reported in the cat.

Malignant Histiocytosis[178-182]

Malignant histiocytosis, a rare condition in cats, is characterized by systemic proliferation of malignant macrophages (histiocytes) and their precursors. A distinguishing characteristic of this disease is erythrophagocytosis. Hepatosplenomegaly with progressive anemia (sometimes Coombs positive) and thrombocytopenia are characteristic. The erythrophagocytosis may be confused with a possible immune-mediated anemia. Bone marrow biopsy, rather than aspiration, and splenic biopsy may be necessary to establish a diagnosis. Special stains using acid phosphatase and nonspecific esterase with fluoride inhibition (naphthol butyrate substrate) may be necessary to indicate macrophage origin.

Myelofibrosis and Myeloid Metaplasia[183-186]

Myelofibrosis and myeloid hyperplasia are characterized by abnormal growth and differentiation of erythroid, myeloid, and megakaryocytic cell types with varying proliferation of fibroblasts in the marrow. Anemia, leukopenia, or thrombocytopenia or varying combinations are common. Myelofibrosis has been diagnosed in FeLV-positive cats and is directly associated with the virus rather than a consequence of myeloproliferative disorders. Myeloid metaplasia may terminate in acute leukemia and therefore may be considered a preleukemic event.

Treatment of Leukemias

Lymphoid leukemia

The use of chemotherapy to treat ALL has been disappointing. A 27% complete response rate has been reported with a COP regimen.[1,10,138,187] In 15 cats treated with COP for ALL, four achieved a CR and 6 cats had a partial response.[1] The median remission was 7 months (range, 1 to 24 months). One report described a short-term remission in a cat with lymphoid leukemia that was treated with a low dose of cytosine arabinoside (10 mg/m^2 given subcutaneously twice daily).[188]

CLL can be treated with chlorambucil (0.2 mg/kg given orally or 2 mg/cat given every other day) and prednisone at a dose of 1 mg/kg given orally daily. Cats with CLL have a better prognosis and survive 1 to 2 years when treated with chlorambucil. As in humans and dogs, treatment can be withheld if the patient has no significant clinical signs and no profound cytopenias. In one study, a cat with CLL remained stable without chemotherapy for over a year.[158]

Nonlymphoid leukemia

The prognoses for acute nonlymphoblastic leukemias generally are very poor, although some exceptions exist. A treatment regimen consisting of a combination of cytosine arabinoside and cyclophosphamide and multiple blood transfusions was effective at inducing a response for 3 months in a cat with acute megakaryocytic leukemia.[176] Hydroxyurea (Hydrea) can be used to treat chronic myeloid leukemia and primary erythrocytosis. Cats with primary erythrocytosis that go untreated are reported to survive 6 to 20+ weeks.[170,172] Phlebotomy alone every 2 to 3 months was used to treat one cat, which survived longer than 20 months.[171] Hydroxyurea treatment for primary erythrocytosis was used in eight cats, and all survived longer than 1 year.[172] Hydroxyurea is available in 500 mg capsules, and the dosage is 25 to 50 mg/kg given orally daily. Some cats have been given 500 mg every 5 to 7 days, but methemoglobulinemia and hemolytic anemia with Heinz bodies has been seen.[172] A better recommendation is to have the drug formulated into 125 mg capsules, which is a more appropriate dosage. However, care must be used in making these capsules because hydroxyurea is potentially carcinogenic. A recommended treatment schedule for hydroxyurea is 125 mg daily to every other day, depending on the type of leukemia under treatment. The drug is tolerated very well at this dosage. A case of acute ML in a cat undergoing treatment with cytarabine, doxorubicin, vincristine, and prednisolone has been reported; a partial remission was noted for approximately 2 months, and the cat survived for 3 months after diagnosis.[147]

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SECTION C
Canine Acute Myeloid Leukemia, Chronic Myeloproliferative Diseases, and Myelodysplasia

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Myeloproliferative diseases are a group of neoplastic diseases of bone marrow marked by unregulated clonal proliferation of hematopoietic stem cells.[1] Excessive proliferation of cells with defective maturation and function leads to reduction of normal hematopoiesis and invasion of other tissues. MPDs have been classified based on the biologic behavior, the degree of cellular differentiation, and the lineage of the neoplastic cells (granulocytic, monocytic, erythroid, megakaryocytic, or mixed), and newer classification systems in humans have incorporated genetics. In 1991 the Animal Leukemia Study Group made recommendations for the classification of nonlymphoid leukemias in dogs and cats.[2] More recently, the Oncology Committee of the American College of Veterinary Pathologists (ACVP) established a Myeloid Neoplasm Subcommittee to re-examine criteria for a classification system and to spearhead large multi-institutional studies to validate the criteria.

Incidence and Risk Factors

MPDs are uncommon or rare in the dog; they occur 10 times less frequently than lymphoproliferative disorders.[3] Accurate information about the incidence and other epidemiologic information await consistent use of a uniform classification system (see later discussion). No known age, breed, or gender predisposition exists, although large breed dogs have been overrepresented in

some retrospective studies.[4-12] In dogs, the etiology of spontaneously occurring leukemia is unknown. Genetic and environmental factors (including exposure to radiation, drugs, or toxic chemicals) probably play a role. In humans, acquired chromosomal derangements lead to clonal overgrowth with arrested development.[13] Chromosomal abnormalities have been reported in dogs with acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and lymphoid leukemia.[14,15] However, because karyotyping is difficult to perform in dogs, owing to the large number and morphologic similarity of their chromosomes and their resistance to banding, definition of the genetic factors in canine MPDs awaits application of molecular techniques and use of the canine genome map.[14,16,17] Certain forms of leukemia in dogs have been produced experimentally by irradiation.[18-20] In contrast to MPDs in cats, no causative viral agent has been demonstrated in dogs, although retrovirus-like budding particles were observed in the neoplastic cells of a dog with granulocytic leukemia.[21]

Pathology and Natural Behavior

A review of normal hematopoiesis can aid the clinician's understanding of the various manifestations of MPDs. Hematopoiesis is the process of proliferation, differentiation, and maturation of stem cells into terminally differentiated blood cells (a simplified schematic is presented in Figure 31-20). Pluripotent stem cells differentiate into either lymphopoietic or hematopoietic multipotent stem cells.[22] Under the influence of specific regulatory and microenvironmental factors, multipotent stem cells in bone marrow differentiate into progenitor cells committed to a specific hematopoietic cell line, such as erythroid, granulocytic-monocytic, or megakaryocytic cells. Maturation results in the production of terminally differentiated blood
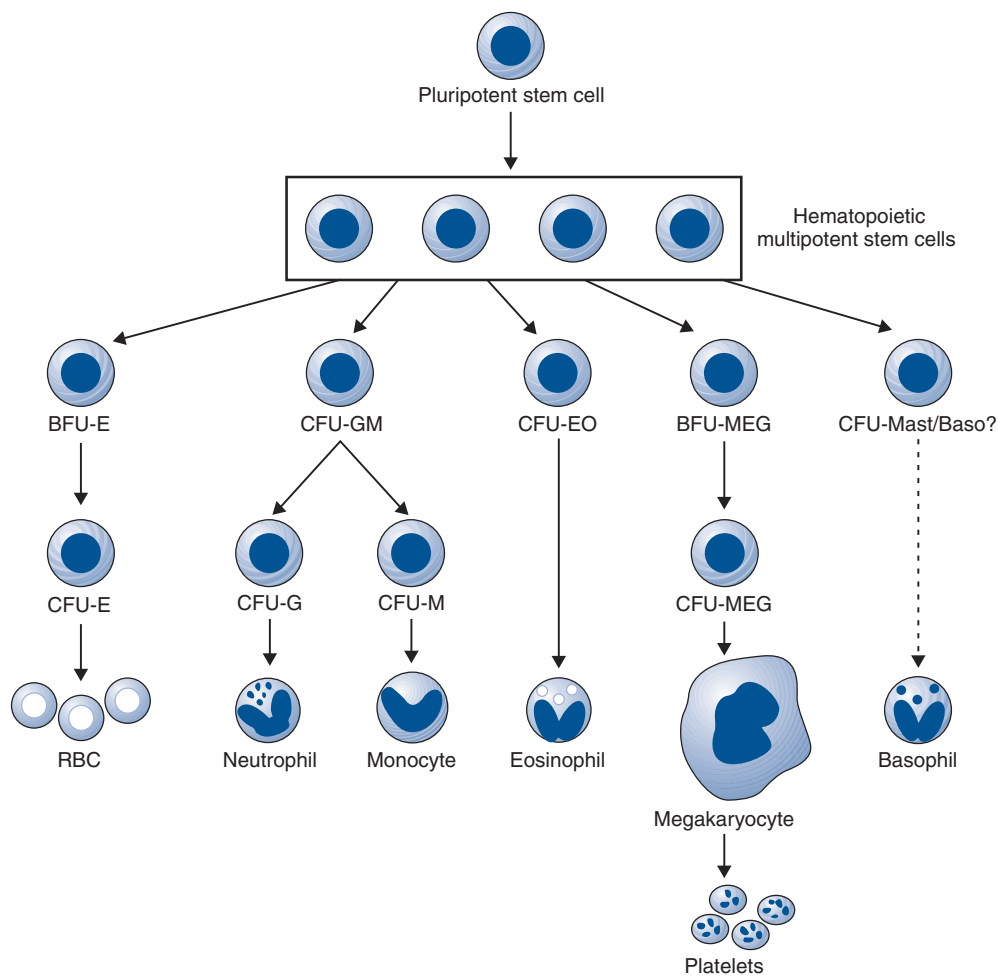
Figure 31-20

Simplified schematic of hematopoiesis. *CFU*, Colony-forming units; *E*, erythroid; *GM*, granulocytic-monocytic; *EO*, eosinophil; *MEG*, megakaryocyte.

cells—erythrocytes, granulocytes, monocytes, and platelets—that are delivered to the circulation. In some cases, as in the maturation of reticulocytes to erythrocytes, final development may occur in the spleen.

The proliferation and differentiation of hematopoietic cells are controlled by a group of regulatory growth factors.[22,23] Of these, erythropoietin is the best characterized; it regulates erythroid proliferation and differentiation and is produced in the kidneys, where changes in oxygen tension are detected. The myeloid compartment depends on a group of factors, collectively referred to as *colony-stimulating factors (CSFs)*. These factors act at the level of the committed progenitor cells but also influence the functional capabilities of mature cells. Some of these factors have a broad spectrum of activity; others are more restricted in their target cells and actions. CSFs are produced *in vitro* by a multitude of cell types, including monocytes, macrophages, lymphocytes, and endothelial cells, and these cells likely play a role in the production and regulation of these factors *in vivo*. The gene for thrombopoietin also has been cloned, and this hormone alone apparently can induce differentiation of megakaryocytes and platelet production.[24] Recombinant forms of many of these hormones are becoming increasingly available.

The clonal disorders of bone marrow include myeloaplasia (usually referred to as *aplastic anemia*), myelodysplasia, and myeloproliferation. A preleukemic syndrome, characterized by peripheral pancytopenia and bone marrow hyperplasia with maturation arrest, is more correctly called *myelodysplasia* because the syndrome does not always progress to overt leukemia. This syndrome has been described in cats, usually in association with FeLV infection, but it has only rarely been recognized in dogs.[25-28] MPDs may be manifested by abnormalities in any or all of the different cell lines, because hematopoietic cells share a common stem cell. In addition, transformation from one MPD to another may occur.[29]

MPDs are classified in several ways. The terms *acute* and *chronic* refer to the degree of cellular differentiation of the leukemic cells, but these terms also correlate with the biologic behavior of the neoplasm.[30] Disorders resulting from uncontrolled proliferation of cells incapable of maturation lead to the accumulation of poorly differentiated, or blast, cells. These disorders have been called *acute MPDs* or *acute nonlymphocytic leukemias*, but they now are included under the umbrella term *acute myeloid leukemia*. Disorders resulting from unregulated proliferation of cells that exhibit progressive, albeit incomplete and defective, maturation lead to the accumulation of differentiated cells. These disorders are called *chronic MPDs*. They include polycythemia vera, CML and its variants, essential thrombocythemia, and possibly primary myelofibrosis. MPDs are further classified by the lineage of the dominant cell type or types, as defined by Romanowsky stains, special cytochemical stains, ultrastructural features, and immunologic cell markers, and they recently have been classified into subtypes (see later discussion).

Acute leukemias have a more sudden onset and are more aggressive. In both acute and chronic disorders, however, abnormalities in proliferation, maturation, and functional characteristics can occur in any hematopoietic cell line.[1] In addition, normal hematopoiesis is adversely affected. Animals with leukemia usually have decreased numbers of circulating normal cells. The pathogenesis of the cytopenias is complex and may result in part from production of inhibitory factors. Eventually, neoplastic cells displace normal hematopoietic cells, a process called *myelophthisis*. Anemia and thrombocytopenia are particularly common. Neutropenia and thrombocytopenia result in infection and hemorrhage, which may be more deleterious to the animal than the primary disease process. Despite the disseminated nature of the disease at the time of diagnosis, parenchymal organ dysfunction usually occurs only in very advanced cases of MPD.

ACUTE MYELOID LEUKEMIA

AML is rare and is characterized by aberrant proliferation of a clone of cells without maturation. This results in the accumulation of immature blast cells in the bone marrow and peripheral blood (Figure 31-21). The WBC count varies, ranging from leukopenia to counts greater than 150,000/μl. The spleen, liver, and lymph nodes commonly are involved, and other tissues may be infiltrated as well, including the tonsils, kidneys, heart, and CNS. No characteristic age has been noted, and even very young dogs may be affected.[31] The clinical course of these disorders tends to be rapid. Production of normal peripheral blood cells usually is diminished or absent, and anemia, neutropenia, and thrombocytopenia are common. Infection and hemorrhage are common sequelae. Occasionally, malignant blasts are present in the bone marrow but not the peripheral blood; this is called *aleukemic leukemia*. *Subleukemic leukemia* suggests a normal or decreased WBC with some neoplastic cells in circulation.

In 1985 the Animal Leukemia Study Group was formed under the auspices of the American Society for Veterinary Clinical Pathology to develop specific morphologic and cytochemical criteria for classifying acute nonlymphocytic leukemias in dogs and cats. Recognition of specific subtypes of leukemia is necessary for the accumulation of accurate, useful information about prognosis and response to treatment and for comparison of studies from different sites. In 1991 this group proposed a classification system following
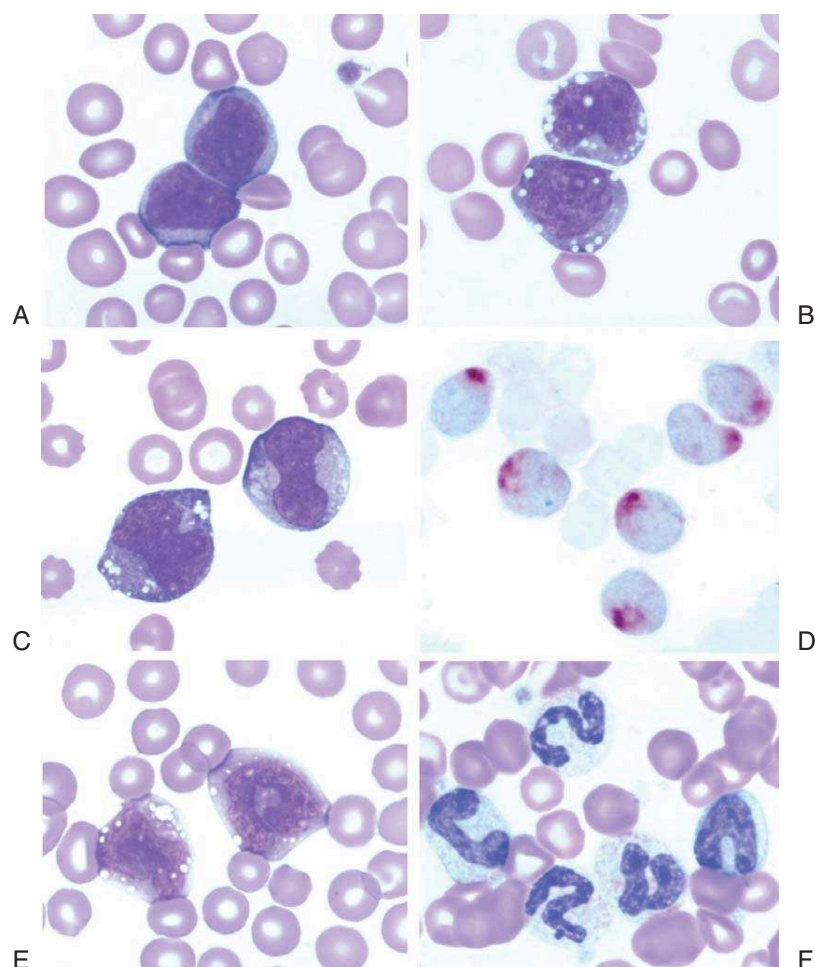
Figure 31-21

Peripheral blood from dogs with myeloproliferative disorders. All diagnoses were confirmed by cytochemical staining. Note how similar the blast cells appear in **A, B,** and **C. A,** Acute myeloblastic leukemia (M1). (Wright's stain, ×100 objective.) **B,** Acute myelomonocytic leukemia (M4). (Wright's stain, ×1 objective.) **C,** Acute monocytic leukemia (M5a). (Wright's stain.) **D,** Acute monocytic leukemia (M5a). (Cytochemical stain [i.e., a-naphthyl butyrate esterase, a nonspecific esterase] with red reaction product.) **E,** Acute monocytic leukemia with some differentiation (M5b). (Wright's stain, ×100 objective.) **F,** Chronic myelogenous leukemia. (Wright's stain, ×100 objective.)

adaptation of the French-American-British (FAB) system and criteria established by the National Cancer Institute Workshop.[2] The group members examined blood and bone marrow from 49 dogs and cats with MPDs. Romanowsky-stained specimens were examined first to identify blast cells and their percentages. Lineage specificity then was determined using cytochemical markers. The percentage of blasts and the information about lineage specificity were used in combination to classify disorders as acute undifferentiated leukemia (AUL), acute myeloid leukemia (AML, subtypes M1 to M5 and M7), and erythroleukemia with or without erythroid predominance (subtypes M6 and M6Er) (see Box 31-7).

Except for acute promyelocytic leukemia (subtype M3), all these subtypes have been described in dogs. However, because this modified FAB system has only recently been adopted, the names given to these disorders in the literature vary considerably. In addition, in the absence of special cytochemical staining, immunophenotyping, and/or electron microscopy, the specific subtype of leukemia often has been uncertain, making retrospective analysis of epidemiologic information, prognosis, and response to therapy confusing at best.

Although defining specific subtypes may seem to be an academic exercise owing to the uniformly poor

prognosis of acute leukemias, this information is critical to improving the management of these diseases. Because of the low incidence of MPDs, national and international cooperative efforts are required to accumulate information on the pathogenesis of specific subtypes and their response to different treatment modalities; use of a uniform classification system is an essential first step.

Different forms of AML are shown in Figure 31-21, *A-E*. The most frequently reported forms of AML in the dog are acute myeloblastic leukemia (M1 and M2) and acute myelomonocytic leukemia (M4).[3-7,19-30,32-39] Megakaryoblastic leukemia (M7) also is well recognized in dogs[8,40-46] and may be associated with platelet dysfunction.[44] Monocytic leukemias likely have included those with and without monocytic differentiation (M5a and M5b),[9,47] but in some cases the diagnosis may have been chronic myelomonocytic or chronic monocytic leukemia (see later discussion). There are few reports in dogs of spontaneously occurring erythroleukemia (M6) in which the leukemic cells comprise myeloblasts, monoblasts, and erythroid elements.[48,49] AULs have uncertain lineages, because they are negative for all cytochemical markers. These leukemias should be distinguished from lymphoid leukemias by flow cytometric analysis of the leukemic cells for cellular antigens that identify their lineage.[50] In addition, examination of blast cells by electron microscopy may reveal characteristic ultrastructural features.

CHRONIC MYELOPROLIFERATIVE DISEASES

Chronic MPDs are characterized by excessive production of differentiated bone marrow cells, which results in the accumulation of erythrocytes (polycythemia vera), granulocytes and/or monocytes (chronic myelogenous leukemia and its variants), or platelets (essential thrombocythemia). Primary myelofibrosis as a clonal disorder of marrow stromal cells, characterized by proliferation of fibroblasts with accumulation of collagen in bone marrow, is not recognized in animals. Myelofibrosis is considered a response to injury and may occur secondary to MPDs.

Polycythemia Vera

Polycythemia vera (PV) is a clonal disorder of stem cells, although whether the defect is in the pluripotent stem cell or the hematopoietic multipotent stem cell is still unclear. In humans, progenitor cells have an increased sensitivity to insulin-like growth factor 1 (IGF-1), which stimulates hematopoiesis.[51] Whether this hypersensitivity is the primary defect or occurs secondary to another gene mutation is unknown. In any case, the result is overproduction of RBCs. The disease is rare and must be distinguished from more common causes of polycythemia, including relative and secondary absolute polycythemia (see later discussion). In PV, neoplastic proliferation of the erythroid series with terminal differentiation to RBCs occurs. The disease has been reported mostly in middle-aged dogs, and no breed or gender predilection has been noted.[52-60] PV is characterized by an increased RBC mass, evidenced by an elevated PCV, RBC count, and hemoglobin concentration. The PCV typically is in the range of 65% to 85%. The bone marrow is hyperplastic, although the M:E tends to be normal. In contrast to the disease in humans, other cell lines do not appear to be involved, and transformation to other MPDs has not been reported. The disease in dogs may be more appropriately called primary erythrocytosis.

Chronic Myelogenous Leukemia

CML is a neoplastic proliferation of the neutrophil series, although concurrent eosinophilic and basophilic differentiation can occur. CML can occur in dogs of any age.[31,61-65] Neutrophils and neutrophilic precursors accumulate in the bone marrow and peripheral blood and invade other organs. The peripheral WBC count usually, but not always, is higher than 100,000/μl. Both immature and mature neutrophils are present (see Figure 31-21, *F*). Mature forms usually are more numerous, but sometimes an "uneven" left shift is present. Signs of dysplasia may be evident, including hypersegmentation, ringed nuclei, and giant forms. Eosinophils and basophils may also be increased. The bone marrow is characterized by granulocytic hyperplasia, and morphologic abnormalities may not be present. Erythroid and megakaryocytic lines may be affected, resulting in anemia, thrombocytopenia or, less commonly, thrombocytosis.

CML must be distinguished from severe neutrophilic leukocytosis and "leukemoid reactions" caused by inflammation or immune-mediated diseases. Leukemoid reactions also can occur as a paraneoplastic syndrome. In humans with CML, characteristic cytogenetic abnormalities are present in all bone marrow cells, signifying a lesion at the level of an early multipotent stem cell. Typically these individuals have a chromosomal translocation, resulting in the Philadelphia chromosome.[66] No consistent cytogenetic abnormalities have been demonstrated in spontaneously occurring CML in dogs. Variants of CML are chronic myelomonocytic leukemia (CMML) and chronic monocytic leukemia (CMoL). These diagnoses are made based on the percentage of monocytes in the leukemic cell population.

Besides accumulating in the bone marrow and peripheral blood, leukemic cells invade the red pulp of

the spleen, the periportal and sinusoidal areas of the liver, and sometimes the lymph nodes. Other organs (e.g., the kidneys, heart, and lungs) are less commonly affected. In addition, extramedullary hematopoiesis may be present in the liver and spleen. Death usually results from complications of infection or hemorrhage secondary to neutrophil dysfunction and thrombocytopenia. In some cases, CML may terminate in "blast crisis," in which a transformation occurs from a predominance of well-differentiated granulocytes to excessive numbers of poorly differentiated blast cells in the peripheral blood and bone marrow; this phenomenon is well documented in the dog.[61,62,64]

Basophilic and Eosinophilic Leukemia

Basophilic leukemia, although rare, has been reported in dogs and is characterized by an elevated WBC count with a high proportion of basophils in the peripheral blood and bone marrow.[67-69] Hepatosplenomegaly, lymphadenopathy, and thrombocytosis may be present, and these dogs have all been anemic. Basophilic leukemia should be distinguished from mast cell leukemia (mastocytosis). Whether dogs develop eosinophilic leukemia remains in question. Reported cases have had high blood eosinophil counts and eosinophilic infiltrates in organs.[70,71] One dog responded well to treatment with corticosteroids. The distinction between neoplastic proliferation of eosinophils and idiopathic hypereosinophilic syndrome remains elusive. Disorders associated with eosinophilia (e.g., parasitism, skin diseases, and diseases of the respiratory and GI tracts) should be considered first in an animal with eosinophilia. One distinguishing feature should be clonality, with reactive eosinophilia comprising polyclonal cells and the neoplastic condition arising from a single clone. As clonality assays become more available, this discrepancy may be resolved.

Essential Thrombocythemia

In humans, essential thrombocythemia, or primary thrombocytosis, is characterized by platelet counts that are persistently higher than $600,000/\mu l$. No blast cells are in circulation, and marked megakaryocytic hyperplasia of the bone marrow without myelofibrosis is present. Thrombosis and bleeding are the most common sequelae, and most patients have splenomegaly. Other MPDs, especially PV, should be ruled out, and the patient should have no primary disorders associated with reactive thrombocytosis,[72] such as inflammation, hemolytic anemia, iron deficiency anemia, malignancies, recovery from severe hemorrhage, rebound from immune-mediated thrombocytopenia, or abscence of a spleen. In addition, certain drugs, such as vincristine, can induce thrombocytosis.

Essential thrombocythemia has been recognized in dogs.[29,73-76] In one dog, the platelet count exceeded 4 million/μl, and bizarre giant forms with abnormal granulation were present. The bone marrow contained increased numbers of megakaryocytes and megakaryoblasts, but circulating blast cells were not seen. Other findings included splenomegaly, GI bleeding, and increased numbers of circulating basophils. Causes of secondary or reactive thrombocytosis were ruled out.[73]

Basophilia also was reported in a more recent case.[75] In another dog, primary thrombocytosis was diagnosed and then progressed to CML.[29] In some cases reported in the literature as essential thrombocythemia, the dogs had microcytic hypochromic anemias. Because iron deficiency anemia is associated with reactive or secondary thrombocytosis, care must be taken to rule out this disorder. However, spurious microcytosis may be reported if a dog has many giant platelets that are counted by an analyzer as small RBCs.[76] Microscopic review of the blood film may be helpful in these cases.

OTHER BONE MARROW DISORDERS

Myelofibrosis

Primary myelofibrosis with clonal proliferation of marrow fibroblasts has not been reported in dogs.[77] In humans, myelofibrosis is characterized by collagen deposition in the bone marrow and increased numbers of megakaryocytes, many of which have morphologic abnormalities. In fact, breakdown of intramedullary megakaryocytes and subsequent release of factors that promote fibroblast proliferation or inhibit collagen breakdown may be the underlying pathogenesis of the fibrosis.[78] Focal osteosclerosis sometimes is present. Anemia, thrombocytopenia, splenomegaly, and myeloid metaplasia (production of hematopoietic cells outside the bone marrow) are consistent features. The extramedullary hematopoiesis is ineffective at maintaining or restoring normal peripheral blood counts.

In dogs, myelofibrosis occurs secondary to MPDs, radiation damage, and congenital hemolytic anemias.[79-82] In some cases the inciting cause is unknown (idiopathic myelofibrosis). Concurrent marrow necrosis may occur in cases of ehrlichiosis, septicemia, or drug toxicity (estrogens, cephalosporins), and some speculate that fibroblasts proliferate in response to release of inflammatory mediators associated with the necrosis.[77] Myeloid metaplasia has been reported to occur in the liver, spleen, and lungs.[82] Extramedullary hematopoiesis is ineffective at preventing or correcting the pancytopenia that eventually develops.

Myelodysplastic Syndrome

Dysfunction of the hematopoietic system can be manifested by a variety of abnormalities that comprise myelodysplastic syndromes (MDS). In dogs, in which the syndrome is rare, cytopenias usually are seen in two or three lines in the peripheral blood (anemia, neutropenia, and/or thrombocytopenia). Other blood abnormalities can include macrocytic erythrocytes and metarubricytosis. The bone marrow typically is normocellular or hypercellular, and dysplastic changes are evident in several cell lines. If blast cells are present, they account for fewer than 30% of all nucleated cells,[2] although this threshold may be altered to fewer than 20%.[16] Myelodysplasia sometimes is called *preleukemia* because in some cases it may progress to an acute leukemia.[25-27] In humans and in cats, MDS are clonal disorders and are considered neoplastic.

History and Clinical Signs

Dogs with MPDs have similar presentations regardless of the specific disease entity, although animals with acute MPDs have a more acute onset of illness and a more rapid clinical course. A history of lethargy, inappetence, and weight loss is common. Clinical signs include emaciation, persistent fever, pallor, petechiation, hepatosplenomegaly and, less commonly, lymphadenopathy and enlarged tonsils. Shifting leg lameness, ocular lesions, and recurrent infections also are seen. Vomiting, diarrhea, dyspnea, and neurologic signs are variable features. Serum chemistries may be within reference intervals but can change if significant organ infiltration occurs.

Animals with MSD may be lethargic and anorectic and may have pallor, fever, and hepatosplenomegaly. In PV, dogs often have erythema of mucous membranes because of the increased RBC mass. Some dogs are polydipsic. In addition, neurologic signs may be present, such as disorientation, ataxia, or seizures, and these are thought to be the result of hyperviscosity or hypervolemia.[56] Hepatosplenomegaly usually is absent.

Peripheral blood abnormalities are consistently found. In addition to the presence of neoplastic cells, other abnormalities may be present, including a decrease in the numbers of normal cells of any or all hematopoietic cell lines. Occasional nucleated RBCs are present in the blood of about half of dogs with acute nonlymphocytic leukemia.[2] Nonregenerative anemia and thrombocytopenia are present in most cases. The anemia usually is normocytic and normochromic, although macrocytic anemia sometimes is present. Pathogenic mechanisms include the effects of inhibitory factors, leading to ineffective hematopoiesis, myelophthisis, immune-mediated anemia secondary to neoplasia, and hemorrhage secondary to thrombocytopenia,

platelet dysfunction, or DIC. Anemia is most severe in acute MPDs, although both anemia and thrombocytopenia may be milder in animals with the M5 subtype (acute monocytic leukemia). In myelofibrosis, the anemia is characterized by anisocytosis and poikilocytosis. In addition, pancytopenia and leukoerythroblastosis, in which immature erythroid and myeloid cells are in circulation may be present. These phenomena probably result from the replacement of marrow by fibrous tissue, with resultant shearing of red cells and escape of immature cells normally confined to bone marrow. In PV, the PCV is elevated, usually in the range of 65% to 85%. The bone marrow is hyperplastic, and the M:E usually is in the normal range.

The neoplastic cells often are defective functionally. Platelet dysfunction has been reported in a dog with acute megakaryoblastic leukemia (M7),[44] and in CML, the neutrophils have decreased phagocytic capacity and other abnormalities. One exception to this was a report of CML in a dog in which the neutrophils had enhanced phagocytic capacity and superoxide production.[83] The authors hypothesized that increased synthesis of granulocyte-macrophage colony-stimulating factor (GM-CSF) resulted from a lactoferrin deficiency in the neoplastic neutrophils and mediated the enhanced function of these cells.

Diagnostic Techniques and Workup

In all cases of MPD, the diagnosis depends on the examination of peripheral blood and bone marrow. Acute MPDs are diagnosed on the basis of finding blast cells with clearly visible nucleoli in blood and bone marrow. Most dogs with acute leukemia have circulating blasts. These cells may be present in low numbers in peripheral blood, and the smear should be searched carefully, especially at the feathered edge. Even if blasts are not detected in circulation, indications of bone marrow disease, such as nonregenerative anemia or thrombocytopenia, usually are present. Occasionally neoplastic cells can be found in CSF in animals with invasion of the CNS. Smears of aspirates from tissues such as the lymph nodes, spleen, or liver may contain blasts but usually contribute little to the diagnostic workup.

Examination of blasts stained with standard Romanowsky stains may give clues to the lineage of the cells (see Figure 31-21, *A* to *C* and *E*). In AML, in addition to myeloblasts, some progranulocytes with their characteristic azurophilic granules may be present. In myelomonocytic leukemia, the nuclei of the blasts usually are pleomorphic and have round to lobulated forms. In some cells the cytoplasm may contain large azurophilic granules or vacuoles. Blasts in megakaryocytic leukemia may contain vacuoles and may have cytoplasmic blebs. In addition, bizarre macroplatelets may be present. Although these distinguishing morphologic

features may suggest a definitive diagnosis, cytochemical stains or immunophenotyping usually is required to define the lineage of the blasts. Several investigators have reported modification of diagnoses after cytochemical staining.[84,85] It is especially important to distinguish AML from lymphocytic leukemia to provide prognostic information to the owner.

The Animal Leukemia Group recommends the following diagnostic criteria (Figure 31-22).[2] Using well-prepared Romanowsky-stained blood and bone marrow films, a minimum of 200 cells are counted to determine the leukocyte differential in blood and the percentage of blast cells in bone marrow or blood or both. In bone marrow, blast cells are calculated both as a percentage of all nucleated cells (ANC) and nonerythroid cells (NEC) and are further characterized using cytochemical markers.[84-86] Neutrophil differentiation is identified by positive staining of blasts for peroxidase, Sudan black B, and chloracetate esterase. Nonspecific esterases (alpha-naphthyl acetate esterase or alpha-naphthyl butyrate esterase), especially if inhibited by sodium fluoride, mark monocytes. Canine monocytes

Figure 31-22

Scheme for classifying myeloid leukemias and myelodysplastic syndromes in dogs and cats. (Blast cells are myeloblasts, monoblasts, and megakaryoblasts.) *ANC*, All nucleated cells in bone marrow, including lymphocytes, plasma cells, macrophages, and mast cells; *NEC*, nonerythroid cells in bone marrow; *AUL*, acute undifferentiated leukemia; *AML*, acute myeloid leukemias M1-M5 and M7; *CML*, chronic myeloid leukemias, including chronic myelogenous, chronic myelomonocytic, and chronic monocytic leukemias; *MDS*, myelodysplastic syndrome; *MDS-Er*, myelodysplastic syndrome with erythroid predominance; *M6*, erythroleukemia; *M6Er*, erythroleukemia with erythroid predominance. *(From Jain NC, Blue JT, Grindem CB et al: Vet Clin Pathol 20:63-82, 1991.)*

may also contain a few peroxidase-positive granules. Acetylcholinesterase is a marker for megakaryocytes in dogs and cats. In addition, positive immunostaining for von Willebrand's factor (factor VIII–related antigen) and platelet glycoproteins on the surface of blasts identifies them as megakaryocyte precursors.[8,42-46] Alkaline phosphatase (AP) only rarely marks normal cells in dogs and cats but is present in blast cells in acute myeloblastic and myelomonocytic leukemias. However, because of reports of AP activity in lymphoid leukemias in dogs, its specificity as a marker for myeloid cells is not certain. Omega-exonuclease is a specific marker for basophils, which are also positive for chloracetate esterase activity.[69]

Blood and bone marrow differential counts and cytochemical staining should be performed and interpreted by experienced veterinary cytopathologists. If erythroid cells comprise less than 50% of ANC and the blast cells account for more than 30%, a diagnosis of AML or AUL is made. If erythroid cells are more than 50% of ANC and the blast cells are more than 30%, a diagnosis of erythroleukemia (M6) is made. If rubriblasts comprise a significant proportion of the blast cells, a diagnosis of M6Er, or erythroleukemia with erythroid predominance, can be made. (It should be noted that in the human AML classification system, the blast threshold has been lowered to 20%.)

In some cases, electron microscopy is required to identify the lineage of the blast cells. For example, megakaryocyte precursors are positive for platelet peroxidase activity and contain demarcation membranes and alpha granules.[42,46] Both these features are detected at the ultrastructural level. Immunophenotyping, used to identify cell lineages in human patients, awaits development of appropriate markers for animal species (see later discussion). Hematopoietic cells from humans with leukemia often have abnormal chromosome patterns. Cytogenetic abnormalities have been found in leukemic cells from a small number of dogs.[14,15] It is not clear whether chromosomal aberrations are primary (causative) or secondary (caused by the leukemia). If consistent karyotypic patterns can be identified and correlated with leukemic subtypes, cytogenetic analysis eventually may yield important diagnostic and prognostic information and become a valuable tool for evaluating remission and predicting relapse.

Although morphologic and cytochemical analyses have formed the mainstay of cell identification, newer technologies now are used routinely to classify leukemias by using monoclonal antibodies to detect antigens associated with certain cell types. Cells can be immunophenotyped using flow cytometric analysis or immunocytochemistry. Both lymphoid and nonlymphoid acute leukemias are positive for CD34. Many lymphocyte markers, including CD3, CD4, CD8, CD18,

CD21, CD45, CD79, and IgG, are available for dogs and can be used to rule out lymphoblastic leukemia in dogs with acute leukemias.[50,87] Other markers include myeloperoxidase (MPO) and CD11b for myeloid cells and CD41 for megakaryoblasts. Some overlap in the expression of these cell antigens occurs. For example, canine (but not human) granulocytes express CD4. It is best to use a panel of antibodies (similar to using a battery of cytochemical stains), because antigens often are expressed on multiple lineages, and lineage infidelity can occur. These tests have become more valuable with the availability of canine reagents. Currently, the Myeloid Neoplasm Subcommittee of the ACVP Oncology Committee recommends that the following immunophenotyping panel be done on bone marrow and/or blood smears to characterize animal leukemias: for B lymphocytes, CD79a; for T lymphocytes, CD3; for myeloid cells, MPO and CD11b; for megakaryoblasts, CD41; for dendritic cells, CD1c; and for acute leukemias, CD34.[16]

Owing to the degree of differentiation of cells in chronic MPDs, these disorders must be distinguished from nonneoplastic causes of increases in these cell types. To allow a diagnosis of PV, tests first must establish that the polycythemia is absolute rather than relative. In relative polycythemias, plasma volume is decreased from hemoconcentration, dehydration, or hypovolemia, and the absolute red cell mass is not increased. Splenic contraction also can result in relative polycythemia. Absolute polycythemia, in which the RBC mass is increased, usually occurs secondary to tissue hypoxia, causing appropriate increased production of erythropoietin. In rare cases, erythropoietin may be produced inappropriately by a tumor (e.g., renal cell carcinoma) or in renal disease (pyelonephritis) or localized renal hypoxia.[88-90] These causes of polycythemia should be eliminated by appropriate laboratory work, thoracic radiographs, arterial blood gas analysis, and renal ultrasonography. In humans with PV, plasma erythropoietin (Epo) levels are low. Epo levels in dogs with PV tend to be low or low-normal, whereas in animals with secondary absolute polycythemia, the levels are high.[91,92] Samples for determination of Epo concentrations should be taken before therapeutic phlebotomy to treat hyperviscosity and, owing to fluctuations in Epo levels, should be repeated if results are incongruous with other information.

CML in dogs has no pathognomonic features, and other common causes for marked leukocytosis with a left shift (leukemoid reaction) and granulocytic hyperplasia of bone marrow must be eliminated. These include infections, especially pyogenic ones; immune-mediated diseases; and other malignant neoplasms. In CML, maturation sometimes appears disorderly, and variation in the size and shape of neutrophils may occur at the same level of maturation. In addition, neoplastic

leukocytes may disintegrate more rapidly and appear vacuolated.[31] Because of the invasive nature of CML, biopsy of the liver or spleen may also help distinguish true leukemia from a leukemoid reaction, assuming the animal can tolerate the procedure. If characteristic cytogenetic abnormalities can be found in dogs with CML, this analysis may be helpful.

Basophilic leukemia is diagnosed by finding excessive numbers of basophils in circulation and in bone marrow. Basophilic leukemia must be differentiated from mastocytosis based on the morphology of the cell type present. Basophils have a segmented nucleus and variably sized granules, whereas mast cells have a round to oval nucleus that may be partly or totally obscured by small, round, metachromatic-staining granules. This distinction usually is easy to make; however, in basophilic leukemia, changes in the morphology of the nucleus and granules make the distinction less clear.[68]

Essential thrombocythemia has been diagnosed based on finding persistent and excessive thrombocytosis (more than $600,000/\mu l$) without circulating blast cells and in the absence of another MPD (e.g., polycythemia vera), myelofibrosis, or disorders known to cause secondary thrombocytosis.[72] These include iron deficiency anemia, chronic inflammatory diseases, recovery from severe hemorrhage, rebound from immune-mediated thrombocytopenia, and absence of a spleen. Thrombocytosis is transient in these disorders or abates with resolution of the primary disease. In essential thrombocythemia, platelet morphology may be abnormal, with bizarre giant forms and abnormal granulation.[73] In the bone marrow, megakaryocytic hyperplasia is a consistent feature, and dysplastic changes may be evident in megakaryocytes.[76] Spurious hyperkalemia may be present in serum samples from dogs with thrombocytosis from any cause because of the release of potassium from platelets during clot formation.[93] Measurement of the potassium in plasma is recommended in these cases and usually demonstrates a potassium concentration within reference interval. Platelet aggregability has been variably reported as impaired[73] or enhanced.[76] In the one dog in which it was measured, the plasma thrombopoietin (Tpo) concentration was normal.[75] Whether Tpo plays a role in essential thrombocythemia or is suppressed by the high platelet mass is unclear. Elucidation of the pathogenesis of this disorder should be aided by the recent cloning of the genes for thrombopoietin and its receptor, the proto-oncogene mpl.[94]

In MDS, abnormalities in two or three cell lines usually are manifested in the peripheral blood as neutropenia with or without a left shift, nonregenerative anemia, or thrombocytopenia. Other changes include macrocytosis and metarubricytosis. The bone marrow typically is normocellular or hypercellular with an increased M:E, and blasts cells, although increased, account for fewer than 30% of the nucleated cells.

This blast threshold may be changed to fewer than 20%, and in a report of 13 dogs with primary or secondary MDS, in all but one dog the blast cell percentage was less than 20%.[95] Dysplastic changes can be detected in any cell line. Dyserythropoiesis is characterized by asynchronous maturation of erythroid cells, typified by large hemoglobinized cells with immature nuclei (megaloblastic change). If the erythroid component is dominant, the MDS is called MDS-Er (see Box 31-7).[2,28] In dysgranulopoiesis, giant neutrophil precursors and abnormalities in nuclear segmentation and cytoplasmic granulation can be seen. Finally, dysthrombopoiesis is characterized by giant platelets and micromegakaryocytes.

Myelofibrosis should be suspected in animals with nonregenerative anemia or pancytopenia; abnormalities in erythrocyte morphology, especially shape; and leukoerythroblastosis. Bone marrow aspiration usually is unsuccessful, resulting in a "dry tap"; this necessitates a bone marrow biopsy taken with a Jamshidi needle.[96] The specimen is processed for routine histopathologic examination and, if necessary, special stains for fibrous tissue can be used. Because myelofibrosis occurs secondary to other diseases of bone marrow (e.g., MPD), chronic hemolytic anemia, or bone marrow necrosis, the clinician should look for a primary disease process.

TREATMENT

Acute Leukemias

Because of the poor response of acute nonlymphocytic leukemias, treatment has been unrewarding to date. However, little information is available on the response of specific subtypes of leukemia to uniform chemotherapeutic protocols, partly because of the rarity of these disease processes and the paucity of cases in the literature. The clinician is advised to contact a veterinary oncologist for advice on new protocols and appropriate management of these cases.

The therapeutic goal is to eradicate leukemic cells and re-establish normal hematopoiesis. Currently, this is best accomplished by cytoreductive chemotherapy, and the agents most commonly used include a combination of cytosine arabinoside and an anthracycline (e.g., doxorubicin or cyclophosphamide), vincristine, and prednisone.* In humans, the introduction of cytosine arabinoside has been the single most important development in the therapy of acute nonlymphocytic leukemia.[99] In dogs, a regimen of 100 to 200 mg/m^2 of cytosine arabinoside, given by slow infusion (over 12 to 24 hours) daily for 3 days and repeated weekly, has been used. Doxorubicin (30 mg/m^2 given intravenously every 2 to 3 weeks) can be administered at intervals alternating with cytosine arabinoside. If remission is achieved, as evidenced by normalization of the hemogram, the COAP protocol (cyclophosphamide, vincristine, cytosine arabinoside, and prednisone), as described for canine lymphoma, could be used as maintenance therapy.[7,97] Another protocol that has been used to treat acute myeloblastic leukemia is presented in Table 31-10.

Regardless of the chemotherapy protocol used, significant bone marrow suppression will develop, and intensive supportive care will be necessary. Transfusions of whole blood or platelet-rich plasma may be required

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*References 7, 10, 25, 34, 97, and 98.

TABLE 31-10	Protocol for Treatment of Acute Myeloproliferative Disorders	
Drug	**Dosage**	**Route**
Induction of remission		
Cytosine arabinoside	100 mg/m^2/day	IV over 60 minutes every 12 hours
6-Thioguanine	40 mg/m^2	PO for 4 days
Doxorubicin	10-15 mg/m^2	IV daily for 3 days
Prednisone	40 mg/m^2	PO divided BID × 7 days
Maintenance		
Cytosine arabinoside	100 mg/m^2/day	SQ or IV once or twice a week
6-Thioguanine	40 mg/m^2	PO twice weekly
Doxorubicin	30 mg/m^2	IV every 3 weeks
Prednisone	20 mg/m^2	PO every other day

From Goldman EE, Graham JC: Clinical diagnosis and management of acute nonlymphoid leukemias and chronic myeloproliferative disorders. In Feldman BF, Zinkl JG, Jain NC, editors; Schalm's veterinary hematology, ed 5, Philadelphia, 2000, Lippincott Williams & Wilkins.

IV, Intravenous; PO, ora;, SQ, subcutaneous.

to treat anemia and thrombocytopenia, and infection should be managed with aggressive antibiotic therapy. Because of the generally poor response, the major thrust of therapy may be to provide palliative supportive care.

Polycythemia Vera

For PV, therapy is directed at reducing the red cell mass. The PCV should be reduced to 50% to 60% or by one sixth of its starting value. Phlebotomies should be performed as needed, with administration of appropriate colloid and crystalloid solutions to replace lost electrolytes; 20 ml of whole blood per kilogram of body weight can be removed at regular intervals.[54] In humans, phlebotomy continues to be the therapeutic approach used most frequently.

Radiophosphorus (^{32}P) has been shown to provide long-term control but can be used only in specialized centers.[100] The chemotherapeutic drug of choice is hydroxyurea, an inhibitor of DNA synthesis. This drug should be administered at an initial dosage of 30 mg/kg for 10 days; the dosage then is reduced to 15 mg/kg given orally daily.[56] The major goal of treatment is to maintain the PCV as close to normal as possible.

Chronic Myelogenous Leukemia

CML is best managed with chemotherapy to control the proliferation of the abnormal cell line and improve the quality of life. Hydroxyurea is the most effective agent for treating CML during the chronic phase.[61,101] The initial dosage is 20 to 25 mg/kg given twice daily. Treatment with hydroxyurea should continue until the leukocyte count falls to 15,000 to 20,000 cells/μl.[61,65,67] The dosage of hydroxyurea then can be reduced by 50% on a daily basis or to 50 mg/kg given biweeky or triweekly. In humans, the alkylating agent busulfan can be used as an alternative.[102] An effective dosage has not been established in the dog, but following human protocols, 0.1 mg/kg/day given orally is administered until the leukocyte count is reduced to 15,000 to 20,000 cells/μl.

Despite response to chemotherapy and control for many months, most dogs with CML eventually enter a terminal phase of disease. In one study of seven dogs with CML, four underwent terminal phase blast crisis.[61] In humans, blast crisis may be lymphoid or myeloid.[103] In dogs, the lineage of blast cells often has not been determined. These dogs have a poor prognosis, and the best treatment to consider, if any, would be that listed in Table 31-10.

Essential Thrombocythemia

Few cases have been reported, but one dog was treated successfully with a combination chemotherapy protocol that included vincristine, cytosine arabinoside, cyclophosphamide, and prednisone.[74] Treatment is controversial in humans because of the lack of evidence that asymptomatic patients benefit from chemotherapy. Patients with thrombosis or bleeding are given cytoreductive therapy. Hydroxyurea is the drug of choice for initially controlling the thrombocytosis.[72]

Myelodysplastic Syndrome

No standard therapeutic regimen exists for MDS. Often, humans receive no treatment if the cytopenias do not cause clinical signs. Transfusions are given when necessary, and patients with fever are evaluated aggressively to detect infections. Growth factors, such as erythropoietin, GM-CSF, granulocyte colony-stimulating factor (G-CSF), and IL-3, are sometimes used in patients who require frequent transfusions to increase their blood cell counts and enhance neutrophil function.[104,105] In one case report, human Epo (100 U/kg given subcutaneously every 48 hours) was administered to a dog with MSD because of profound anemia. The rationale for the erythropoietin was to promote terminal differentiation of dysplastic erythrocytes. The PCV increased from 12% to 34% by day 19 of Epo treatment. This dog remained in remission for longer than 30 months.[28] Other factors that induce differentiation of hematopoietic cells include retinoic acid analogs,[106] 1,25 dihydroxyvitamin D_3,[107] interferon-alpha, and conventional chemotherapeutic agents, such as 6-thioguanine and cytosine arabinoside.[108] The propensity of these factors to enhance progression to leukemia is not known in many cases, but the potential risk exists.

PROGNOSIS

In general, the prognosis for animals with chronic MPDs is better than for dogs with acute MPDs, for which the prognosis is grave. The prognosis for PV and chronic myelogenous leukemia is guarded, but significant remissions have been achieved with certain therapeutic regimens and careful monitoring. Animals commonly survive a year or longer.[61,67] The development of blast crisis portends a grave prognosis.

COMPARATIVE ASPECTS

The pathophysiology and therapy of nonlymphocytic leukemia in humans is being studied intensively. The MPDs have been demonstrated to be clonal, with abnormalities evident in all hematopoietic cell lines. Leukemogenesis likely is caused by mutation or amplification of proto-oncogenes in a two-step process that initially involves a single cell and is followed by additional

chromosomal alterations that may involve oncogenes.[1,13] These alterations are manifested as chromosomal abnormalities. Environmental factors known to cause leukemia are exposure to high-dose radiation, benzene (chronic exposure), and alkylating agents.[109] New classification systems have incorporated genetic mutations, more accurately reflect prognoses, and facilitate use of consistent categorization among institutions.[110]

Therapeutic modalities under investigation or development include combination chemotherapy, immunotherapy, cytokine therapy, drug-resistance modulators, proapoptotic agents, antiangiogenic factors, signal transduction-active agents, and bone marrow transplantation. The prognosis for chronic MPDs is better than for acute MPDs. For acute nonlymphocytic leukemias, the prognosis is better for children than adults; only 10% of adults who receive chemotherapy maintain remissions for longer than 5 years.[109] The spontaneous canine diseases probably occur too infrequently to serve as useful models. MPDs have been induced experimentally in the dog by irradiation and transplantation in an attempt to create models for study. Many similarities between human and canine MPDs exist, and veterinary medicine may benefit from any therapeutic advances made in the human field.

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SECTION D
Plasma Cell Neoplasms

David M. Vail

Plasma cell neoplasms arise when a cell of the B-lymphocyte plasma cell lineage proliferates to form a malignant population of similar cells. This population is believed in most instances to be monoclonal (i.e., derived from a single cell), because the cells typically produce homogenous immunoglobulin, although some examples of biclonal and polyclonal plasma cell neoplasms exist. A wide variety of clinical syndromes are represented by plasma cell neoplasms, including multiple myeloma, IgM (Waldenstrom's) macroglobulinemia, and solitary plasmacytoma (including solitary osseous plasmacytoma and extramedullary plasmacytoma). Based on incidence and severity, multiple myeloma is the most clinically important plasma cell neoplasm.

MULTIPLE MYELOMA

Incidence and Etiology

Although multiple myeloma (MM) represents fewer than 1% of all malignant tumors in animals, it accounts for approximately 8% of all hematopoietic tumors and 3.6% of all primary and secondary tumors affecting

bones in dogs.[1,2] Early studies indicated a male predisposition,[3] but subsequent reports do not suggest a gender predilection for the dog.[1,4] Older dogs are affected most often (average age, 8 to 9 years).[1,3,4] In one large case series, German shepherds were overrepresented based on the hospital population.[1]

The true incidence of MM in the cat is unknown, although it is a much rarer diagnosis in that species than in the dog. MM accounted for only one of 395 and four of 3248 tumors in two large compilations of feline malignancies and 0.9% of all malignancies and 1.9% of hematologic malignancies in another report.[5-7] MM occurs in aged cats (median age, 12 to 14 years), most often in domestic shorthairs, and no gender predilection has been consistently reported, although a male preponderance may exist.[4,7,8] MM has not been associated with coronavirus, FeLV or FIV infection.

The etiology of MM for the most part is unknown. Genetic predispositions, molecular aberrations, viral infections, chronic immune stimulation, and exposure to carcinogen stimulation all have been suggested as contributing factors.[4,8-14] Support for a familial association in cats comes from cases reported among siblings.[8] Evidence exists that molecular mechanisms of cellular control, including overexpression of cell cycle control components such as cyclin D1(see Chapter 2) and receptor tyrosine kinase dysregulation, may be involved in canine myeloma and plasma cell tumors.[13,14] In rodent models, chronic immune stimulation and exposure to implanted silicone gel has been associated with development of MM,[9,10] as have chronic infections and prolonged hyposensitization therapy in humans.[11] Viral Aleutian disease of mink results in monoclonal gammopathies in a small percentage of cases.[12] Working in the agricultural industry, petroleum products, and irradiation are known risk factors for the development of MM in humans.[15-17] Progression of solitary plasma cell tumors to MM has been reported in both dogs and cats, and a single case of a B-cell lymphoma that progressed to MM has been reported in the dog.[18,19]

Pathology and Natural Behavior

MM is a systemic proliferation of malignant plasma cells or their precursors, which arise as a clone of a single cell that usually involves multiple bone marrow sites. Malignant plasma cells can have a varied appearance on histologic sections and cytologic preparations. The degree of differentiation ranges from cells that resemble normal plasma cells in late stages of differentiation (Figure 31-23) to very large, anaplastic round cells with a high mitotic index, representing early stages of differentiation.[3,4,7,20]

In cats with MM, most plasma cells (83% in one report) are immature and have marked atypia, including increased size, multiple nuclei, clefted nuclei, anisocytosis,
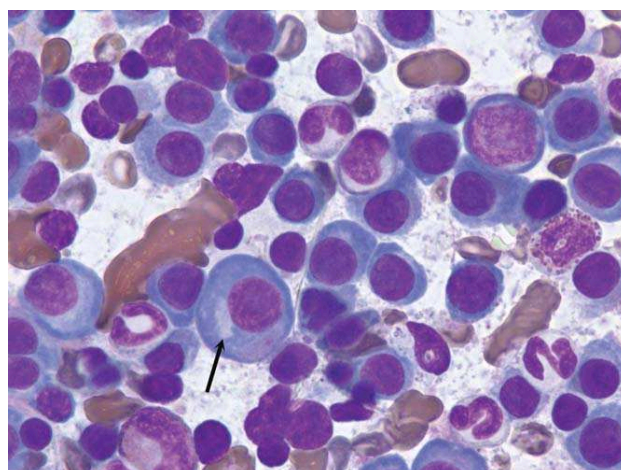
Figure 31-23

Bone marrow aspirate from a dog with multiple myeloma, showing an overabundance of large, neoplastic plasma cells with the characteristic perinuclear clear zone, which represents the Golgi apparatus *(arrow)*. (Diff-Quik stain, ×100 objective.)

anisokaryosis, variable nucleus-to-cytoplasm ratios, decreased chromatin density, and variable nucleoli. Nearly one fourth of the cells have "flame cell" morphology, characterized by peripheral eosinophilic cytoplasmic processes.[7]

Malignant plasma cells typically produce an overabundance of a single type or component of immunoglobulin, referred to as the *M component* (Figure 31-24). The M component may be represented by any class of the entire immunoglobulin or by only a portion of the molecule, such as the light chain (Bence Jones protein) or the heavy chain (heavy chain disease). In the dog, the M component usually is represented by either IgG or IgA immunoglobulin types in nearly equal incidence, whereas the ratio of IgG to IgA in cats is approximately 5:1.* If the M component is the IgM type, the term *macroglobulinemia* (also Waldenstrom's macroglobulinemia) often is applied. Several cases of biclonal gammopathy in dogs and cats have been reported.[7,8,26-30] Two cases of nonsecretory MM have been reported in dogs.[31] Rarely, cryoglobulinemia has been reported in dogs with MM and IgM macroglobulinemia, and it has been reported in a cat with IgG myeloma.[4,32-34] Cryoglobulins are paraproteins that are insoluble at temperatures below 37° C. They require blood collection and clotting to be performed at 37° C before serum separation. If whole blood is allowed to clot at temperatures below this, the protein precipitates

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*References 1, 3, 4, 6, 7, and 21-25.

Normal Pooled Canine Serum

electrophoretic strip

A

Patient Serum

electrophoretic strip

B
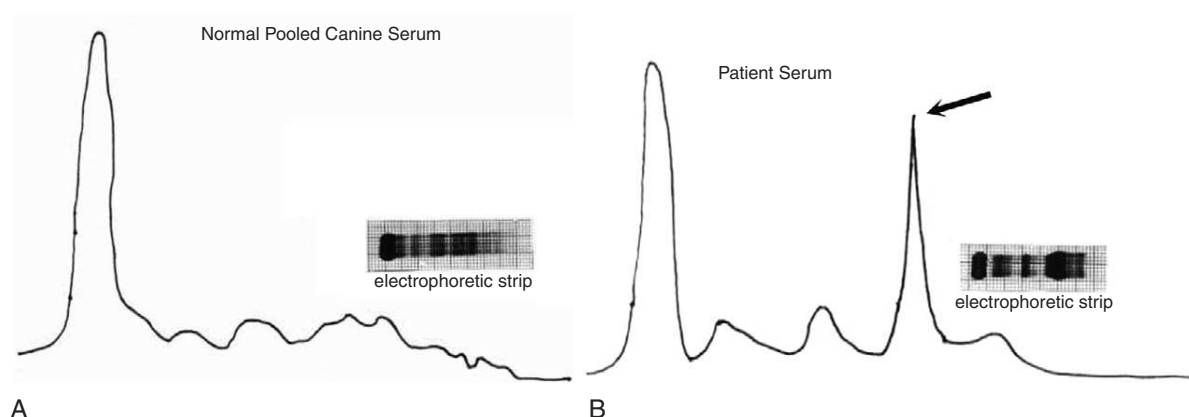
Figure 31-24

A, Serum protein electrophoresis from pooled normal dog serum (stained cellulose acetate electrophoretic strip with accompanying densitogram). **B,** Serum protein electrophoresis from a dog with multiple myeloma. Note the large M component spike (indicating an IgA monoclonal gammopathy) in the gamma region (*arrow*).

in the clot and is lost. Pure light-chain M component is rare but has been reported in dogs.[35]

The pathology associated with MM is a result of high levels of circulating M component or of organ or bone infiltration with neoplastic cells, or both. Associated pathologic conditions include bone disease, bleeding diathesis, hyperviscosity syndrome, renal disease, hypercalcemia, immunodeficiency (and subsequent susceptibility to infections), cytopenias secondary to myelophthisis, and cardiac failure.

Bone lesions can be isolated, discrete osteolytic lesions (including pathologic fractures [Figure 31-25, *A*]) or diffuse osteopenias (Figure 31-26). Approximately one fourth to two thirds of dogs with MM have radiographic evidence of bony lysis or diffuse osteoporosis.[1,3,4,36]

Skeletal lesions in cats with MM were rarely reported in the older literature, but more recent reports document lesions in 50% to 65% of cases.[6-8,22] Bones engaged in active hematopoiesis are more commonly affected, including the vertebrae, ribs, pelvis, skull, and proximal or distal long bones.[3]

Skeletal lesions are rare with IgM (Waldenstrom's) macrogammaglobulinemia, in which malignant cells often infiltrate the spleen, liver, and lymph tissue rather than bone.[4,33,37-39]

Bleeding diathesis can result from one or a combination of events. M components may interfere with coagulation by inhibiting platelet aggregation and the release of platelet factor 3; causing adsorption of minor clotting proteins; generating abnormal fibrin polymerization; and producing a functional decrease in calcium.[4,40-42] Approximately one third of dogs and one fourth of cats have clinical evidence of hemorrhage.[1,7,8] In dogs, nearly half have abnormal prothrombin (PT) and partial

thromboplastin (PTT) times. Thrombocytopenia may also play a role if bone marrow infiltration is significant (i.e., myelophthisis).

Hyperviscosity syndrome (HVS) represents one or a constellation of clinicopathologic abnormalities resulting from greatly increased serum viscosity. The magnitude of viscosity changes depends on the type, size, shape, and concentration of the M component in the blood. HVS is more common with IgM macroglobulinemias because of the high molecular weight of this class of immunoglobulin.[37] IgA myelomas, usually present as a dimer in the dog, may undergo polymerization, resulting in increased serum viscosity.[1,4,40] IgG-associated HVS also can occur, albeit less frequently. High serum viscosity occurs in approximately 20% of dogs with MM and can result in bleeding diathesis, neurologic signs (e.g., dementia, depression, seizure activity, and coma), ophthalmic abnormalities (e.g., dilated and tortuous retinal vessels, retinal hemorrhage [Figure 31-27], and retinal detachment), and increased cardiac workload with the potential for subsequent development of cardiomyopathy.* These consequences are thought to be a result of sludging of blood in small vessels, ineffective delivery of oxygen and nutrients, and coagulation abnormalities. HVS was reported less commonly in cats with MM in the older literature but has been reported in association with IgG-, IgA-, and IgM-secreting tumors.[4,6,21,23-25] In four of nine cats with MM, the relative serum viscosity was above control ranges.[8]

Renal disease is present in approximately one third to one half of dogs with MM, and azotemia was observed

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*References 1, 4, 37, 39, 40, and 43-45.

Figure 31-25

A, Radiograph of a distal femur in a dog with severe osteolysis and a pathologic fracture secondary to a plasma cell tumor. **B,** Radiograph of the same pathologic fracture after surgical repair with Rush rods and bone cement. The local site was treated with adjuvant radiation. The dog was continued on chemotherapy for 2 more years and did well.

in one third of cats in one report.[1,3,7] The pathogenesis of renal failure often is multifactorial. It can develop as a result of Bence Jones (light-chain) proteinuria, tumor infiltration into renal tissue, hypercalcemia, amyloidosis, diminished perfusion secondary to hyperviscosity syndrome, dehydration, or ascending urinary tract infection.[1,4,40-42] Normally, heavy-chain and light-chain synthesis is well balanced in nonneoplastic immunoglobulin production. In MM, an unbalanced excess of light-chain products may result.[42] Light chains have a low molecular weight and normally are filtered by the renal glomerulus; their presence can result in protein precipitates and subsequent renal tubular injury. The presence of light chains in urine without a concomitant monoclonal spike in serum, although rare, is indicative of pure light-chain disease.[35] Tubules become obstructed by large laminated casts containing albumin, immunoglobulin, and light chains.[4,35,40-42] Bence Jones proteinuria occurs in approximately 25% to 40% of dogs with MM, and in two recent surveys, it occurred in approximately 50% of cases.*

Hypercalcemia is reported in 15% to 20 % of dogs with MM and is thought to result primarily from the production of osteoclast-activating factor by neoplastic cells.[1,4,46] Other factors have been implicated in human MM, including elevated levels of various cytokines, TNF, IL-1, and IL-6. In two dogs with MM and hypercalcemia, serum elevations in circulating N-terminal PTHrP were noted.[47] Hypercalcemia may also be exacerbated by associated renal disease. Initially thought to be a rare event in cats with MM, hypercalcemia was noted in 25% of recently reported cases.[7,8,48]

Susceptibility to infection and immunodeficiency long have been associated with MM and often are the ultimate cause of death in affected animals.[1,4,22]

*References 1, 3, 4, 6-8, 22, and 24.
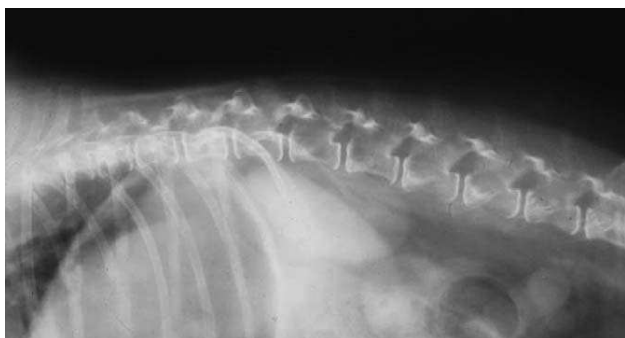
Figure 31-26

Lateral vertebral radiographs of a dog with diffuse osteopenia secondary to multiple myeloma. Note the overall decreased opacity of the lumbar vertebrae, occurring secondary to diffuse marrow involvement and resulted in loss of bone trabeculae and thinning of the cortices. *(Courtesy Dr. Lisa Forrest, University of Wisconsin–Madison).*

TABLE 31-11	Frequency of Clinical Signs for Dogs with Multiple Myeloma*
Clinical Sign	**Frequency Reported (%)**
Lethargy and weakness	62
Lameness	47
Bleeding diathesis	37
Funduscopic abnormalities	35
Polyuria/polydipsia	25
Central nervous system (CNS) deficits	12

From Matus RE, Leifer CE, MacEwen EG et al: Prognostic factors for multiple myeloma in the dog, J Am Vet Med Assoc 188:1288-1291, 1986.
N = 60 dogs.

Figure 31-27

Multiple retinal hemorrhages on the fundus in a cat with hyperviscosity syndrome secondary to multiple myeloma.

Infection rates in humans with MM are 15 times higher than normal and usually represent pneumonia or a urinary tract infection.[49] The response to vaccination also has been shown to be suppressed in humans with MM.[50] Normal immunoglobulin levels often are severely depressed in affected animals.[4] In addition, leukopenias may develop secondary to myelophthisis.

Variable cytopenias may be observed in association with MM. Approximately two thirds of dogs with MM have a normocytic, normochromic, nonregenerative anemia[1,3,4]; this can result from marrow infiltration (myelophthisis), blood loss from coagulation disorders, anemia of chronic disease, or increased erythrocyte destruction secondary to high serum viscosity. In dogs with MM, similar factors lead to thrombocytopenia in nearly one third of the dogs and leukopenia in nearly one fourth. In cats, approximately two thirds are anemic, one half are thrombocytopenic, and one third are neutropenic.[7,8]

Cardiac disease, if present, usually is a result of excessive cardiac workload and myocardial hypoxia secondary to hyperviscosity.[40,41] Myocardial infiltration with amyloid and anemia may be complicating factors. In one report nearly half of the cats with MM had a cardiac murmur, for which the etiology was not established.[7]

History and Clinical Signs

Clinical signs of MM may be present up to a year before diagnosis in dogs (median, 1 month). In one cat, M-component elevations were detected 9 years before clinical presentation.[1,7] In the latter case, the M-component elevation was consistent with monoclonal gammopathy of unknown significance (MGUS). MGUS (i.e., "benign," "essential," or "idiopathic" monoclonal gammopathy) is a benign monoclonal gammopathy that is not associated with osteolysis, bone marrow infiltration, or Bence Jones proteinuria. MGUS also has been reported in dogs.[51,52]

Signs of MM can vary, depending on the wide range of possible pathologic effects. Tables 31-11 and 31-12 list the relative frequencies of clinical signs observed in the dog and cat, respectively, based on a compilation of

TABLE 31-12	Approximate Frequency of Clinical Signs for Cats with Multiple Myeloma*	
Clinical Sign	**Frequency Range Reported (%)**
Lethargy and weakness	60-100
Anorexia	55-100
Pallor	30-100
Polyuria/polydipsia	20-40
Vomiting/diarrhea	20-30
Dehydration	20-30
Organomegaly	20-25
Lameness	10-25
Heart murmur	0-45
Hind limb paresis/paralysis	0-45
Bleeding diathesis	0-40
Central nervous system (CNS) dementia	0-30
Lymphadenopathy	0-10
Cocurrent cutaneous plasma cell tumor	0-10

From MacEwen EG, Hurvitz AI: Diagnosis and management of monoclonal gammopathies, Vet Clin North Am Small Anim Pract 7:119-132, 1977; Patel RT, Caceres A, French AF et al: Multiple myeloma in 16 cats: a retrospective study, Vet Clin Pathol 34:341-352, 2005; Hanna F: Multiple myelomas in cats, J Feline Med Surg 7:275-287, 2005; and Drazner FH: Multiple myeloma in the cat, Comp Cont Ed Pract Vet 4:206-216, 1982.

*N = 36 cats.

several reports.[1,4,7,8,22] Bleeding diathesis usually is represented by epistaxis and gingival bleeding. Funduscopic abnormalities may include retinal hemorrhage (see Figure 31-27), venous dilation with sacculation and tortuosity, retinal detachment and blindness.[1,4,40,43-45] CNS signs may include dementia, seizure activity, and deficiencies in midbrain or brainstem localizing reflexes secondary to HVS or extreme hypercalcemia. Signs reflecting transverse myelopathies secondary to vertebral column infiltration, pathologic fracture, or extradural mass compression also can occur.[1,4,32,36,53] One case of ataxia and seizure activity in a dog with extramedullary plasmacytoma (EMP) secondary to tumor-associated hypoglycemia has been reported.[54] In addition, paraneoplastic polyneuropathy has been reported in a dog with MM.[55]

A history of chronic respiratory infections and persistent fever may also be present in cats. Hepatosplenomegaly and renomegaly can occur as a result of organ infiltration. Bleeding diathesis due to HVS is less common in the cat, but epistaxis, pleural and peritoneal hemorrhagic effusions, retinal hemorrhage, and central neurologic signs have been reported.[4,6,8721-25] Polydipsia and polyuria can occur secondary to renal disease or hypercalcemia, and dehydration may develop. Hind limb paresis secondary to osteolysis of lumbar vertebral bodies or extradural compression has been reported in cats.[8,56]

Diagnostics and Staging

The diagnosis of MM usually follows the demonstration of bone marrow plasmacytosis (see Figure 31-23), the presence of osteolytic bone lesions (see Figure 31-26), and the demonstration of serum or urine myeloma proteins (M component; see Figure 31-24). In the absence of osteolytic bone lesions, a diagnosis can also be made if marrow plasmacytosis is associated with a progressive increase in the M component. In the cat, because the degree of bone marrow infiltration may not be as marked, some have suggested consideration of plasma cell morphology and visceral organ infiltration in cases with demonstrable M-component disease in the absence of marked marrow plasmacytosis (less than 20%).[7]

All animals suspected of having plasma cell tumors should receive a minimal diagnostic evaluation, including a CBC, platelet count, serum biochemistry profile, and urinalysis. Particular attention should be paid to renal function and serum calcium levels. Serum electrophoresis and immunoelectrophoresis are performed to detect a monoclonal spike (see Figure 31-24) and to categorize the immunoglobulin class involved. Heat precipitation with electrophoresis of urine is performed to detect Bence Jones proteinuria, because commercial urine dipstick methods are not capable of this determination.

Definitive diagnosis usually is done by bone marrow aspiration. A bone marrow core biopsy or multiple aspirates may be necessary because of the possibility of clustering of plasma cells in the bone marrow. Normal marrow contains fewer than 5% plasma cells, whereas in myelomatoid marrow, this level often is greatly exceeded. Current recommendations require the presence of marrow plasmacytosis greater than 20%; however, a 10% cutoff in cats recently has been recommended, with special attention to cellular atypia.[7] Patel and colleagues[7] comment that even the 10% threshold is problematic, and cellular atypia and visceral organ involvement should be considered equally important.

Imaging

Routine thoracic and abdominal radiographs are recommended. Occasionally, bony lesions can be observed in skeletal areas on these standard films, and organomegaly (liver, spleen, and kidneys) is observed in most cats (Figure 31-28).[7] Abdominal ultrasound scans are recommended in all cats suspected of having MM, because they reveal one or more abdominal tumors in nearly all such patients.[7] These include splenomegaly with or without
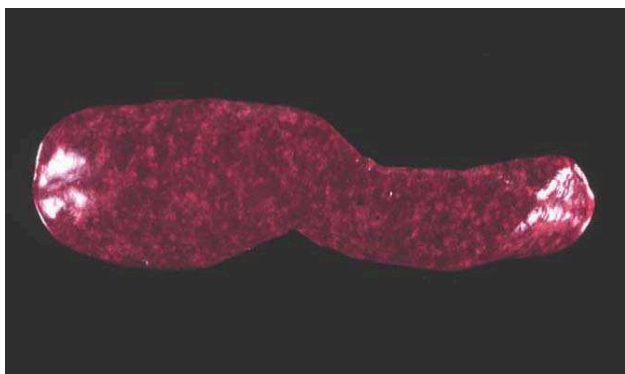
Figure 31-28

Necropsy specimen of a spleen from a cat with multiple myeloma showing diffuse myeloma infiltration.

nodules, diffuse hyperechoic hepatomegaly with or without nodules, renomegaly, and iliac lymph node enlargement. Skeletal survey radiographs are recommended to detect and determine the extent of osteolytic lesions, which may have diagnostic, prognostic, and therapeutic implications. Nuclear scintigraphy (bone scans) for clinical staging of dogs with MM has been performed; however, because of the predominant osteolytic activity with osteoblastic inactivity, these scans seldom give positive results and therefore are not useful for routine diagnosis.[57] In physician-based oncology, bone mineral density analysis (DEXA scan) to document osteoporosis and MRI scans of bone marrow are commonly used for staging; however, neither of these has been applied consistently in the veterinary literature.

In rare cases, biopsy of osteolytic lesions (i.e., Jamshidi core biopsy; see Chapter 23) is necessary for diagnosis. In one case of MM in a dog, splenic aspirates were diagnostically helpful.[58] If clinical hemorrhage is present, a coagulation assessment (e.g., platelet count, PT, and PTT) and serum viscosity measurements should be done. All animals should undergo a careful funduscopic examination. Table 31-13 shows the overall frequency of clinical diagnostic abnormalities in dogs and cats with MM, as compiled from published series involving at least five cases each.

A clinical staging system for canine MM has been suggested[1]; however, currently no prognostic significance has been attributed to it.

Molecular diagnostic techniques for MM have had limited use thus far in veterinary oncology; however, PCR techniques have been used to determine the clonality of the immunoglobulin heavy-chain variable region gene in feline plasmacytomas and myelomas (see Section A of this chapter and Figure 31-8).[59] The use of this technology in cases in which the diagnosis is not straightforward awaits further investigation.

Differential diagnosis of multiple myeloma

Disease syndromes other than plasma cell tumors can be associated with monoclonal gammopathies and should be considered in any list of differentials. These include other lymphoreticular tumors (lymphoma, extramedullary plasmacytoma, chronic and acute lymphocytic leukemia), chronic infections (e.g., ehrlichiosis, leishmaniasis, FIP), and MGUS.*

Treatment

Initial therapy

Therapy for MM is directed at both the tumor cell mass and the secondary systemic effects. All diagnostic procedures should be completed before primary therapy is started to ensure a complete diagnosis and to procure baseline values for monitoring response.

In most dogs with MM, chemotherapy is effective at reducing the myeloma cell burden, relieving bone pain, allowing for skeletal healing, and reducing levels of serum immunoglobulins. It also can greatly extend both the quality and duration of most patients' lives.[1,4] Complete elimination of neoplastic myeloma cells is rare, but the chemotherapeutic drugs currently available can make MM a gratifying disease to treat for both the clinician and the client. However, eventual relapse is to be expected.

Only one half of cats with MM respond to chemotherapy, and most responses are short-lived. However, several long-term responses (i.e., longer than 1 year) have been reported, and treatment should be attempted when educated clients decide on a therapeutic option.†

Melphalan, an alkylating agent, is the chemotherapeutic drug of choice for the treatment of MM.[1,4] In the dog, an initial starting dosage of 0.1 mg/kg is given orally once daily for 10 days and then reduced to 0.05 mg/kg given orally once daily continuously. Addition of prednisone therapy is thought to increase the efficacy of melphalan therapy. Prednisone is started at a dosage of 0.5 mg/kg given orally once daily for 10 days and is then reduced to 0.5 mg/kg given orally every other day until the drug is discontinued after 60 days of therapy. Melphalan, however, is continued at 0.05 mg/kg/day until clinical relapse occurs or myelosuppression necessitates a dose reduction. Most dogs treated with this melphalan/prednisone combination tolerate the regimen well. The most clinically significant toxicity of melphalan is myelosuppression, particularly a delayed thrombocytopenia. A CBC, including a platelet count, should be performed biweekly for 2 months of therapy

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*References 3, 7, 29, 51, 52, and 60-63.
†References 4, 7, 8, 21, 22, and 30.

TABLE 31-13	Approximate Frequency of Clinical Diagnostic Abnormalities in Dogs and Cats with Multiple Myeloma*	
	FREQUENCY RANGE REPORTED (%)	
Abnormality	**Dogs**	**Cats**
Elevated M component	100	94
Monoclonal	100	77
Biclonal	0[†]	23
IgG	46	84
IgA	54	16
Noncutaneous extramedullary extension	Not reported (NR)	100[‡]
Marrow plasmacytosis (>10%)	100	97
Complete blood count (CBC) abnormalities		
Anemia (nonregenerative)	68	64
Thrombocytopenia	33	50
Neutropenia	25	37
Circulating plasma cells (leukemia)	10	25
Hypoalbuminemia	65	36
Hypocholesterolemia	NR	68
Proteinuria	35	71
Bence Jones proteinuria	38	59
Bone lysis	51	45
Serum hyperviscosity	32	44
Azotemia	33	22
Hypercalcemia	16	25
Elevated liver enzymes	NR	43

From Matus RE, Leifer CE, MacEwen EG et al: Prognostic factors for multiple myeloma in the dog, J Am Vet Med Assoc 188:1288-1291, 1986; MacEwen EG, Hurvitz AI: Diagnosis and management of monoclonal gammopathies, Vet Clin North Am Small Anim Pract 7:119-132, 1977; Patel RT, Caceres A, French AF et al: Multiple myeloma in 16 cats: a retrospective study, Vet Clin Pathol 34:341-352, 2005; Hanna F: Multiple myelomas in cats, J Feline Med Surg 7:275-287, 2005; and Drazner FH: Multiple myeloma in the cat, Comp Cont Ed Pract Vet 4:206-216, 1982.

*N = 36 cats and 82 dogs.

[†]Several single case reports exist of biclonal gammopathy in dogs with multiple myeloma.

[‡]All 11 cats in one report had evidence of infiltration in either the spleen, lymph node, or liver.

and monthly thereafter. If significant myelosuppression occurs (usually thrombocytopenia or neutropenia), the dosage or the treatment frequency may need to be reduced.

The author has successfully used an alternative, pulse-dosing regimen for melphalan (7 mg/m^2 given orally daily for 5 consecutive days every 3 weeks) in a small number of cases in which myelosuppression was limiting the more conventional, continuous low-dose therapy. The author now uses this pulse-dose regimen as a first-line treatment with the caveat that long-term response data are lacking.

Melphalan/prednisone therapy also can be used in cats with MM; however, the protocol appears to be more myelosuppressive in cats than in dogs, and careful monitoring is required. In the cat, a dosing schedule similar to that for the dog has been reported[8,22];

0.1 mg/kg (approximately 0.5 mg, or ¼ of a 2 mg tablet) is given orally once daily for 10 to 14 days, then every other day until clinical improvement occurs or leukopenia develops. One report has advocated long-term continuous maintenance (0.1 mg/kg given orally once every 7 days).[8]

Cyclophosphamide has been used as an alternative alkylating agent or in combination with melphalan in dogs with MM.[1,4,64] No evidence indicates that it is superior to melphalan therapy. In the author's practice, cyclophosphamide is limited to patients with severe hypercalcemia or widespread systemic involvement in which a faster acting alkylating agent may alleviate systemic effects more quickly. Cyclophosphamide is initiated at a dosage of 200 mg/m^2 given intravenously once, at the same time oral melphalan therapy is started. Because cyclophosphamide is less likely to affect thrombocytes, it may be substituted

in patients in which thrombocytopenia has developed secondary to long-term melphalan use.

Chlorambucil, another alkylating agent, has been used successfully for the treatment of IgM macroglobulinemia in dogs at a dosage of 0.2 mg/kg given orally once daily.[4,37] Few or no clinical signs of toxicity result from this dosing schedule.

CCNU, yet another alkylating agent, has been used in a limited number of cats with MM, and a partial response has been reported with a dosing schedule of 50 mg/m^2 given orally every 21 days.[65]

Evaluation of the response to therapy

Evaluation of the response to systemic therapy for MM is based on improvement in clinical signs and clinicopathologic parameters and radiographic improvement of skeletal lesions.[1,4] Subjective improvement in clinical signs of bone pain, lameness, lethargy, and anorexia should be evident within 3 to 4 weeks after initiation of therapy. Objective laboratory improvement, including reduction in serum immunoglobulin or Bence Jones proteinuria, usually is noted within 3 to 6 weeks. Radiographic improvement in osteolytic bone lesions may take months, and only partial resolution may occur. Ophthalmic complications (including long-standing retinal detachment) and paraneoplastic neuropathies can be expected to resolve along with the tumor mass.[45,55] In one report on cats that responded to melphalan and prednisone, clinical improvement was noted in 4 weeks and serum protein and radiographic bone abnormalities were greatly improved by 8 weeks.[8]

As previously discussed, MM does not resolve completely, and a good response is defined as a reduction in measured M component (i.e., immunoglobulin or Bence Jones proteins) by at least 50% of pretreatment values.[4] Reduction in the serum immunoglobulin levels may lag behind reduction in Bence Jones proteinuria, because the half-lives are 15 to 20 days and 8 to 12 hours, respectively.[66] For routine follow-up, quantification of the elevated serum immunoglobulin or urine Bence Jones protein is performed monthly until a good response is noted and then every 2 to 3 months. Repeat bone marrow aspiration for evaluation of plasma cell infiltration occasionally may be necessary. This is particularly prudent when cytopenias develop during chemotherapy and drug toxicity must be differentiated from marrow recurrence.

Therapy directed at complications of multiple myeloma

Long-term control of complications such as hypercalcemia, HVS, bleeding diathesis, renal disease, immunosuppression, ophthalmic complications, and pathologic skeletal fractures is achieved by controlling the primary tumor mass. However, therapy directed more specifically at these complications may be indicated in the short term.

If hypercalcemia is marked and significant clinical signs are present, standard therapies, including fluid diureses with or without pharmacologic agents (e.g., calcitonin), may be indicated (see Chapter 5). Moderate hypercalcemia typically resolves within 2 to 3 days after initiation of melphalan/prednisone chemotherapy.

HVS is best treated in the short term by plasmapheresis.[4,40,62,67,68] Whole blood is collected from the patient and centrifuged to separate plasma from packed cells. Packed red cells are resuspended in normal saline and reinfused into the patient. Bleeding diathesis usually resolves along with HVS, but platelet-rich plasma transfusions may be necessary with thrombocytopenia.

Renal impairment may require aggressive fluid therapy in the short term and maintenance of adequate hydration in the long term. Careful attention to secondary urinary tract infections and appropriate antimicrobial therapy are indicated. Ensuring an adequate water intake at home is important, and in some cases owners must be taught home subcutaneous fluid administration. Continued monitoring of renal function is recommended, along with follow-up directed at tumor response.

Patients with MM can be thought of as immunologic cripples. Some have recommended prophylactic antibiotic therapy in dogs with MM,[4] but in humans, no benefit for this approach has been observed over diligent monitoring and aggressive antimicrobial management when indicated.[41] Cidal antimicrobials are preferred over static drugs, and nephrotoxic antimicrobials should not be used.

Pathologic fractures of weight-bearing long bones and of vertebrae, resulting in spinal cord compression, may require immediate intervention in conjunction with systemic chemotherapy. Orthopedic stabilization of fractures should be performed and may be followed with external beam radiotherapy (see Figure 31-25, *B*). Recently, inhibition of osteoclast activity by bisphosphonate drugs has been shown to reduce the incidence and severity of skeletal complications of MM in humans.[57] This class of drugs may hold promise for use in dogs and cats with various skeletal tumors.[69]

Rescue therapy

Rescue therapy may be attempted when MM eventually relapses in dogs undergoing melphalan therapy or in the uncommon patient that initially is resistant to alkylating agents. The author has had success with the VAD protocol, a combination of doxorubicin (30 mg/m^2 given intravenously every 21 days), vincristine (0.7 mg/m^2 given intravenously on days 8 and 15), and dexamethasone sodium phosphate (1 mg/kg given intravenously once a week on days 1, 8, and 15); this regimen is used in 21-day cycles. Although most dogs initially respond to this rescue protocol, the duration of response tends to

Figure 31-29

Survival curve for 37 dogs with multiple myeloma treated with chemotherapy. The median survival time was 540 days. *(From Matus RE, Leifer CE, MacEwen EG et al: J Am Vet Med Assoc 188:1288-1292, 1986.)*

be short, lasting only a few months. High-dose cyclophosphamide (300 mg/m^2 given intravenously every 7 days) also been has used as a rescue agent, although with limited success. Liposomal doxorubicin produced a long-term remission in a dog with MM that had been resistant to native doxorubicin.[70]

Investigational therapies

Because MM ultimately is a uniformly fatal disease in most species, including humans, significant effort is being put into investigational therapies for this disease. Currently, bone marrow ablative therapy and marrow or stem cell rescue, thalidomide (and other antiangiogenic therapies), bortezomib (a proteasome inhibitor), arsenic trioxide, the bisphosphonates, and several molecular targeting therapies are under investigation; however, their use in veterinary species is limited or completely absent at present.[57] Nonetheless, the promise of molecular-targeted therapies is foreshadowed by a case of a dog with MM that was resistant to melphalan, prednisone, and doxorubicin[14]; this dog achieved a partial response to tyrosine kinase inhibitor therapy (SU11654; see Chapter 14, section B) that was maintained for 6 months.

Prognosis

The prognosis for dogs with MM is good for initial control of the tumor and a return to good quality of life. In a group of 60 dogs with MM, approximately 43% achieved a complete remission (i.e., normalization of serum immunoglobulins), 49% achieved a partial

remission (i.e., immunoglobulin levels less than 50% pretreatment values), and only 8% did not respond to melphalan/prednisone chemotherapy.[1] Long-term survival is the norm, with a median of 540 days reported (Figure 31-29). Hypercalcemia, Bence Jones proteinuria, and extensive bony lysis are known negative prognostic indices in the dog.[1] The long-term prognosis for dogs with MM is poor, because recurrence of the tumor mass and associated clinical signs is expected. Eventually, the tumor no longer responds to available chemotherapeutic drugs, and death follows from renal failure, sepsis, or euthanasia for intractable bone or spinal pain.[1,4]

The prognosis for MM in the cat is not as favorable in the short term as it is in the dog.[4,7,8,22] Although most cats (approximately 60%) transiently respond to melphalan/prednisone- or cyclophosphamide-based protocols, most responses are partial and not durable. Typically, cats with MM succumb to the disease within 4 months.[6-8,22,24,68] However, long-term survivors (longer than 1 year) have been reported.[7,8,22,30,48] One investigator grouped MM in cats into two prognostic categories, based on criteria known to predict disease behavior in dogs (Table 31-14).[8] Although no rigorous statistical analysis was performed on this small group of cats (nine), the median survival times were 5 days for cats with disease categorized as "aggressive" and 387 days for those with disease categorized as "nonaggressive."

Experience in dogs with IgM macroglobulinemia is limited.[4,35] Response to chlorambucil is to be expected, and in nine treated dogs, 77% achieved remission, with a median survival time of 11 months.[4]

TABLE 31-14	Classification of Multiple Myeloma in Cats Based on Clinical and Diagnostic Criteria Suspected of Predicting the Prognosis
Tumor Behavior	**Criteria**
Aggressive	Hypocalcemia, presence of bony lesions with pathologic fracture, low packed cell volume (PCV), presence of light-chain Bence Jones protein in urine, azotemia, hypercreatinemia, persistence of high serum protein level after 8 weeks of treatment, little or no clinical improvement
Less aggressive	Normal serum calcium; normal creatinine, blood urea nitrogen (BUN), and PCV levels; presence of bony lesions without pathologic fractures; absence of light-chain Bence Jones protein; normalization of serum protein level after 8 weeks of treatment

From Hanna F: Multiple myelomas in cats, J Feline Med Surg 7:275-287, 2005.

Figure 31-30

Cutaneous plasmacytoma on the limb of a dog.

SOLITARY AND EXTRAMEDULLARY PLASMACYTIC TUMORS

Solitary collections of monoclonal plasmacytic tumors can originate in soft tissues (extramedullary plasmacytoma) or bone (solitary osseous plasmacytoma [SOP]). A number of large case compilations of cutaneous plasmacytoma have been reported in the dog.[13,71-77] Plasmacytomas represented 2.4% of all canine tumor submissions in one large compilation of cases.[78] In this series of 751 cases, the most common locations in the dog were cutaneous sites (86%) (Figure 31-30), the mucous membranes of the oral cavity and lips (9%) (Figure 31-31), and the rectum and colon (4%). The skin of the limbs and head (including the ears) is the most frequently reported cutaneous site.[71,72,77] All other sites accounted for the remaining 1% of cases; these sites may include the stomach, spleen, genitalia, eyes, uterus, and liver. The American cocker spaniel, English cocker spaniel, and West Highland white terrier (and perhaps Yorkshire terriers, boxers, German shepherds, and Airedale terriers) have an increased risk of developing plasmacytomas, and the median age of affected dogs is 9 to 10 years.[77,78]

Cutaneous and oral EMP in dogs typically manifests as benign tumors that are highly amenable to local therapy. The natural behavior of noncutaneous/nonoral EMP appears to be somewhat more aggressive in the dog. Gastrointestinal EMP has been reported in a number of sites in the veterinary literature, including the esophagus,[79] stomach (Figure 31-32),[64,80] small intestine,[81] and large intestine.[78,80,81-83] Metastasis to associated lymph nodes is more common in these cases; however, bone marrow involvement and monoclonal gammopathies are less commonly encountered. In nine cases of colorectal EMP, only two dogs experienced recurrence after surgical excision, and two cases involved multiple lesions.[78] EMP of the trachea, liver, and uterus also have been reported in a dog, and all had a benign course after local resection.[84-86]

Most cases of SOP eventually progress to systemic MM; however, the time course from local tumor development to systemic MM may be many months to years.[31,87] SOPs reported in the dog have involved the zygomatic arch and the ribs.[31]

Plasmacytomas are less common in cats, and fewer reports exist in the literature.[18,88-94] They occur in older cats (mean age, 8.5 years) with no significant gender predilection. The skin is the most common site; other sites include the oral cavity, eye, GI tract, liver, subcutaneous tissues, and brain. Two reports exist of cutaneous EMP in cats that progressed to systemic disease; one cat developed lymph node and distant metastasis, the other progressed to MM.[18,91]

A   B
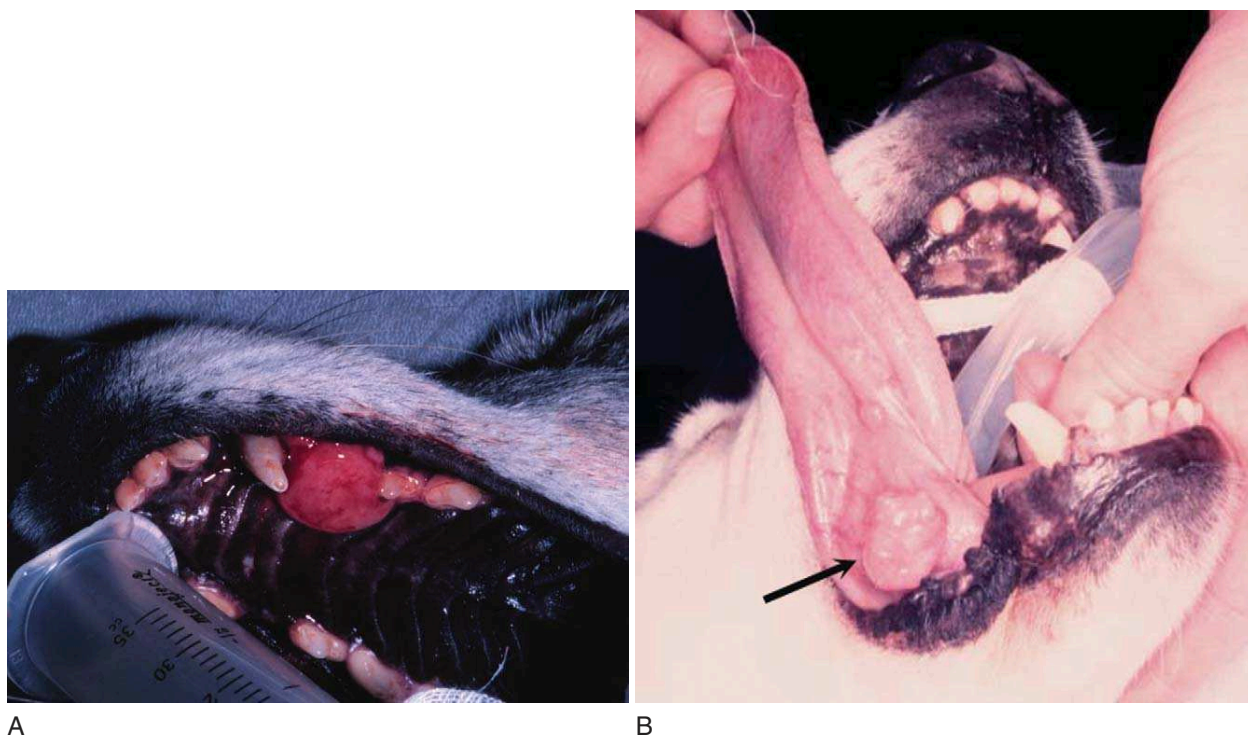
Figure 31-31

Examples of oral solitary plasmacytoma in dogs. **A,** Plasmacytoma involving the maxilla. **B,** Plasmacytoma involving the underside of the tongue. Surgical excision was curative in both dogs.

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Please refer to the printed publication.

Figure 31-32

Endoscopic appearance of an extramedullary plasmacytoma in a dog's stomach. *(From Matus RE, Leifer CE, MacEwen EG et al: J Am Vet Med Assoc 188:1288-1292, 1986.)*

Clinical Signs

Clinical signs associated with solitary plasmacytomas relate to the location of involvement; in the rare cases involving high levels of M component, HVS may occur. Most cutaneous plasmacytomas are solitary, smooth, raised pink nodules that measure 1 to 2 cm in diameter (see Figure 31-30), although tumors as large as 10 cm have been reported. Combination of data from large series shows that more than 95% of the tumors occur as solitary masses, and fewer than 1% occur as part of a systemic MM process.[13,71-73,77] The cutaneous and oral forms of EMP usually have a benign course and no related clinical signs. Gastrointestinal EMP, however, typically produces relatively nonspecific signs that may suggest alimentary involvement. Colorectal plasmacytomas usually cause rectal bleeding, hematochezia, tenesmus, and rectal prolapse.[78] One case of ataxia and seizure activity in a dog with EMP secondary to tumor-associated hypoglycemia has been reported.[54] SOP usually is associated with pain and lameness (if the appendicular skeleton is affected) or with neurologic signs (if vertebral bodies are involved).

Diagnosis

Diagnosis of SOPs and EMPs usually requires tissue biopsy or fine-needle aspiration. The cells that make up

solitary plasmacytic tumors in both cats and dogs have been classified histologically as mature, hyaline, cleaved, asynchronous, monomorphous blastic, and polymorphous blastic cell types. No prognostic significance has been observed with this classification system, although it has been suggested that the polymorphous blastic type may act more aggressively in the dog.[13,77,88]

With poorly differentiated solitary plasmacytic tumors, immunohistochemical studies directed at detecting immunoglobulin, light and heavy chains, and thioflavine T may be helpful for differentiating the lesions from other round cell tumors.[31,76,77,92-94] Immunoreactivity has been demonstrated for canine IgG F(ab)$_2$ and vimentin.[73] A variant characterized by an IgG-reactive amyloid interspersed with the neoplastic cells also has been described.[74] In addition, PCR techniques can be used to determine the clonality of the immunoglobulin heavy-chain variable region gene in plasmacytomas and myelomas, which may be diagnostically useful in difficult cases.

Thorough staging is important in dogs and cats with plasmacytomas that are at higher risk for systemic spread. Staging, which must be done before therapy is started, should include bone marrow aspiration, serum electrophoresis, and skeletal survey radiographs to ensure that the disease is confined to a local site. This is most important for SOP and gastrointestinal EMP because of these tumors' relatively high metastatic rate; it is less important for cutaneous and oral plasmacytomas because of their typically more benign behavior. In addition, endoscopic evaluation of the entire GI tract is recommended with gastrointestinal EMP.

Treatment

Cutaneous plasma cell tumors in the dog are almost always benign and have an excellent prognosis after conservative surgical excision. Successful therapy with melphalan and prednisone has been rarely applied for a local recurrence or incomplete margins in dogs and cats.[53,92] Radiation therapy has been used infrequently for cases that are nonsurgical. Surgery is recommended in combination with radiotherapy for SOP when the lesion results in an unstable long bone fracture (see Figure 31-25) or the patient is nonambulatory because of neurologic compromise caused by a vertebral body SOP. In the latter case, spinal cord decompression, mass excision, and possibly spinal stabilization may be necessary.[53] Radiotherapy can be used without surgery, when fractures are stable, as a palliative measure for bone pain or, in the case of vertebral SOP, if the patient is ambulatory and stable. Good local control usually is achieved, but most patients eventually develop systemic MM.[31,53,87] SOP of the axial skeleton can be managed by excision or radiotherapy alone.

Whether systemic chemotherapy should be initiated at the time of local therapy for SOP when systemic involvement is not documented is the subject of debate. Systemic spread may not occur for many months or even years beyond the primary SOP diagnosis in humans and dogs, and studies in humans reveal no benefit from initiation of systemic chemotherapy before documentation of subsequent systemic spread.[42,53] Similarly, EMP of the GI tract in humans most often is treated with surgical excision and thorough staging of disease. Systemic therapy is not initiated unless systemic involvement is documented. Systemic chemotherapy has been used following excision of a gastric EMP in a cat, but the utility of adjuvant therapy in the species is unkown.[94]

Long-term follow-up of patients with a solitary plasmacytoma is indicated so that recurrence of disease and systemic spread can be recognized. In patients with SOP, careful attention should be paid to the serum globulin levels, bone pain, and the radiographic appearance of bone healing. Restaging of the disease, including bone marrow evaluation, is indicated if systemic spread is suspected.

Prognosis

The prognosis for solitary plasma cell tumors generally is good. Cutaneous and mucocutaneous plasmacytomas usually are cured by surgical excision.[13,77] In large compilations of cases in dogs, the local recurrence rate was approximately 5%, and nodal or distant metastasis occurred in only seven of 349 cases (2%).[13,71-73,77] New cutaneous plasmacytomas at sites distant from the primary tumor developed in fewer than 2% of cases. One caveat: the author has encountered a handful of cases of aggressive multiple cutaneous plasmacytoma that eventual resulted in the death of the affected dogs. Neither the tumor cell proliferation rate (as measured by Ki-67 immunohistochemistry) in the dog nor histopathologic grading in dogs and cats was prognostic in large compilations of cases, although it has been suggested that the polymorphous blastic type may act more aggressively in the dog.[13,77,88] The presence of amyloid and overexpression of cyclin D1 (prognostic in human plasmacytomas) were not shown to be prognostic in dogs.[13]

Most dogs with EMP of the alimentary tract and other abdominal organs (e.g., liver, uterus) that is treated by surgical excision alone or in combination with systemic chemotherapy (if metastasis is present) can enjoy long-term survival.[31,64,78-80,82-86] In a compilation of nine dogs with colorectal plasmacytoma, two had local recurrence at 5 and 8 months after surgery; after surgery alone, the overall median survival time was 15 months.[78] DNA ploidy and c-myc oncoprotein expression in biopsy samples were determined to be predictive for EMPs in dogs; however, tumors that were malignant all were from noncutaneous sites (i.e., lymph node, colon, and spleen), therefore location appears to be as predictive.[96] As previously discussed,

most patients with SOP eventually develop systemic disease, but long, disease-free periods usually precede the event.

The prognosis in cats is less well defined, owing to the scarcity of reported cases. If disease is confined to a local site and/or regional nodes, surgical excision and chemotherapy can result in long-term control; however, early, widespread metastasis and progression to MM is also reported in cats.*

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Miscellaneous Tumors

Hemangiosarcoma

Douglas H. Thamm

INCIDENCE AND RISK FACTORS

Hemangiosarcoma (HSA), also known as malignant hemangioendothelioma or angiosarcoma, is a malignant neoplasm of vascular endothelial origin. Hemangiosarcoma occurs more frequently in dogs than in any other species.[1,2] It represents about 5% of all noncutaneous primary malignant neoplasms and 12% to 21% of all mesenchymal neoplasms in the dog.[3-5] Hemangiosarcoma accounts for 2.3% to 3.6% of skin tumors in dogs,[1,6] and 45% to 51% of splenic malignancies.[2,7,8] It is less common in the cat, occurring in approximately 0.5% of cats examined at necropsy in one study and accounting for 2% of neoplasms.[9,10]

Hemangiosarcoma is seen mostly in middle-aged to older animals, although there are reports in dogs less than 3 years of age.[7,10-14] German shepherds, golden retrievers, and Labrador retrievers are overrepresented in many case series.[7,11,14,15] There may be a slight male predisposition in dogs.[11,12,14,15]

Although the etiology is unknown, reports in humans have been related to exposure to thorium dioxide, arsenicals, vinyl chloride, and androgens.[16] The human vascular neoplasm Kaposi's sarcoma is causally associated with human herpes virus 8, and while some studies have demonstrated herpes viral elements in human angiosarcoma as well, the majority have not.[17-20] There is a documented increase in HSA development in beagle dogs exposed to ionizing radiation pre- or postnatally.[21] Cutaneous HSAs are found more frequently in dogs with minimal pigmentation and thin hair coats[22] and have been associated with ultraviolet light exposure in laboratory dogs.[23]

There is increasing evidence that dysregulation of molecular pathways governing angiogenesis may be important in the pathogenesis of HSA. Studies in humans and dogs have demonstrated abundant expression of the angiogenic growth factors vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoietin-1 (Ang-1) in HSA cells and tissues, and concomitant expression of the cellular receptors for VEGF, bFGF, and Ang-1. This suggests the potential for autocrine stimulation of one or more of these receptors leading to dysregulated proliferation and survival.[24-32] Indeed, enforced overexpression of VEGF is sufficient to transform immortalized murine endothelial cells into HSA,[33] and *in vivo* overexpression of VEGF has led to vascular tumor formation in mice.[34]

Mutations in the von Hippel-Lindau (VHL) tumor suppressor gene, which regulates the cellular response to hypoxia by controlling the abundance of the transcription factor hypoxia-inducible factor-1 (Hif-1) and upregulates the expression of angiogenic growth factors such as VEGF, have been shown to dramatically increase the risk of development of hemangioblastomas and other tumors in humans.[35] Additionally, upregulation of Hif-1 and its downstream target genes have been demonstrated in a human angiosarcoma.[36] Mice lacking one copy of the VHL gene have a greatly increased incidence of vascular neoplasia, including HSA.[37] Mutations in other tumor suppressor genes, such as p53,[38-40] PTEN,[41] Ras,[42-44] and Tsc2[45] have likewise been implicated in the pathogenesis of HSA, based on murine, human, or limited canine studies.

PATHOLOGY AND NATURAL BEHAVIOR

The most common primary site in the dog is the spleen (Figure 32-1).[1,11,12,14,15] Other frequent sites include the right atrium, skin and subcutis, and liver.[11,14,22,46-48] Cases have also been reported in the lung, kidneys, oral cavity, muscle (Figure 32-2), bone, urinary bladder, left ventricle (Figure 32-3), uterus, and retroperitoneum.[12,14,49-52] In the cat, cutaneous (Figure 32-4) and visceral (e.g., spleen, liver, intestine) locations are evenly distributed.[10,53] Other reported sites in the cat
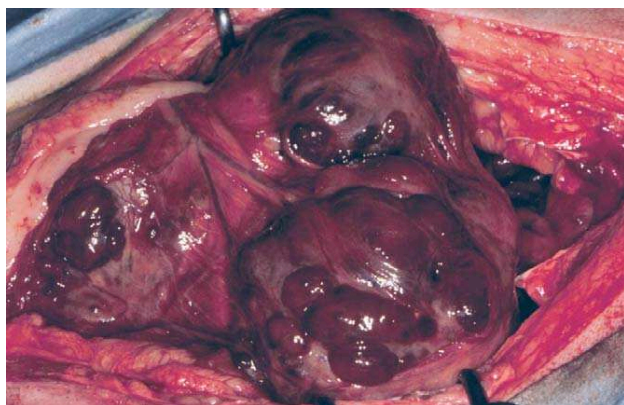
Figure 32-1

Intraoperative image of multifocal hemangiosarcoma within the spleen of a dog. *(Photo courtesy of M.G. O'Brien.)*
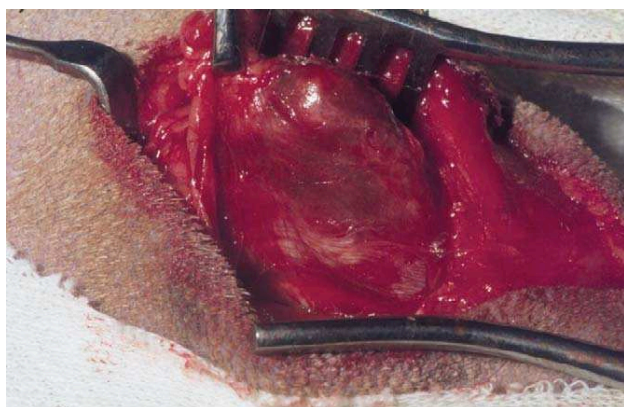
Figure 32-2

Intraoperative image of hemangiosarcoma within the gastrocnemius muscle of a dog. *(Photo courtesy of M. G. O'Brien.)*
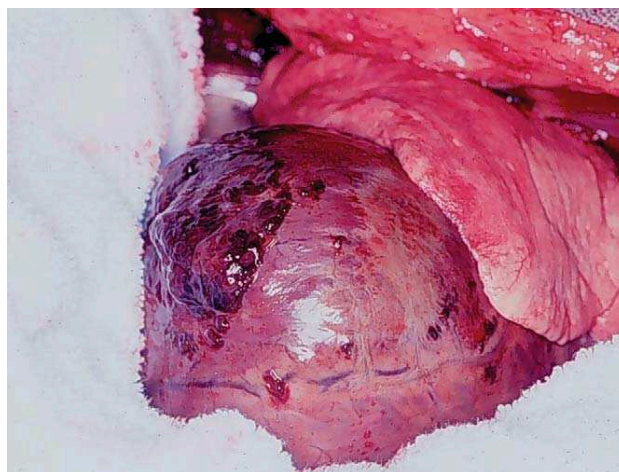
Figure 32-3

Image of a dog presenting with left ventricular hemangiosarcoma with pericardial hemorrhage and acute tamponade.

Figure 32-4

Image of a cat presenting with cutaneous HSA of the periocular tissues. This cat had a partial response to Doxil (liposomal doxorubicin therapy).

include the heart, thoracic cavity, and nasal cavity.[10,53,54] Hemangiosarcoma is the most common splenic neoplasm encountered, but it is by no means the only differential for splenomegaly or splenic masses in dogs. The "double two thirds rule" has been applied to canine splenic masses: approximately two thirds of dogs with splenic masses will have a malignant tumor, and approximately two thirds of those malignancies will be HSA.[2,8,55] However, one study found that approximately 70% of dogs with splenic masses presenting with nontraumatic hemoabdomen were HSA.[56] Several differentials for solitary splenic masses (e.g., HSA,

hematoma, hemangioma, other sarcomas) can have a similar gross and ultrasonographic appearance. Histopathology is necessary to establish a definitive diagnosis in all cases.

Hemangiosarcoma may be solitary, multifocal within an organ, or widely disseminated at presentation. Grossly, they are of variable size, pale gray to dark red or purple, and soft or gelatinous, often containing blood-filled or necrotic areas on cut surface (see Figure 32-1). They are poorly circumscribed, nonencapsulated, and often adhered to adjacent organs. They are extremely friable, and complications associated with rupture and

hemorrhage are frequent presenting complaints. Histologically, HSA consists of immature, pleomorphic endothelial cells forming vascular spaces containing variable amounts of blood or thrombi.[57] In cases where these features are minimal but HSA is suspected, immunohistochemistry for von Willebrand's factor (factor VIII-related antigen) or CD31/PECAM can be used to demonstrate endothelial derivation and support the diagnosis of HSA.[58-60]

Perhaps owing to its intimate association with the vasculature, facilitating extravasation and angiogenesis of metastatic clones, canine HSA is typified by very aggressive biologic behavior with rapid and widespread metastasis being common. An exception to this rule is pure cutaneous or dermal HSA without any clinical or histologic evidence of subdermal infiltration.[47] Metastasis is typically hematogenous or through trans-abdominal implantation following rupture. The most frequent metastatic sites are the liver, omentum, mesentery, and lungs.[14,61] One study suggested that approximately 25% of dogs with splenic HSA will also have right atrial involvement.[61] However, the author's experience suggests that this percentage at initial presentation is significantly lower (15% or less). Other reported sites of metastasis are the kidney, muscle, peritoneum, lymph nodes, adrenal glands, brain, and diaphragm. In dogs, HSA is considered the most common metastatic sarcoma to the brain. In one study of 85 dogs with HSA, 12 (14%) had brain metastasis.[62]

Hemangiosarcoma in cats is considered to be a less aggressive disease. Invasive cutaneous or subcutaneous HSA usually behaves in a fashion similar to other soft-tissue sarcomas, with local recurrence being the major concern.[58] Reports do exist of more aggressive biologic behavior in a subset of cats with cutaneous HSA.[63] Visceral HSAs in cats have a higher metastatic rate, similar to that in dogs, and the common sites include the liver, omentum, diaphragm, pancreas, and lung.[10,53]

HISTORY AND CLINICAL SIGNS

Presenting complaints vary depending on the location of the primary tumor and can range from vague, nonspecific signs of illness, to asymptomatic abdominal swelling, to acute death secondary to hemorrhagic/hypotensive shock. Common presenting complaints for visceral HSA are acute weakness or collapse. These complaints may have been preceded by similar transient episodes occurring over a period of weeks or days, which resolved spontaneously in 12 to 36 hours. In such cases, it is theorized that an HSA rupture leads to hemoperitoneum, followed by subsequent reabsorption (i.e., autotransfusion) of red blood cells. For dogs that present asymptomatically or with vague signs (lethargy, inappetance, weight loss,

abdominal distension), an abdominal mass is often palpated during examination. Dogs with cardiac HSA typically present with signs related to pericardial tamponade and associated right-sided heart failure, such as exercise intolerance, dyspnea, and ascites.

Common physical examination findings for visceral HSA include pale mucous membranes with delayed capillary refill, tachycardia with poor pulse quality, and a palpable fluid wave in the abdomen with or without a palpable abdominal mass. Dogs with cardiac HSA may display ascites, muffled heart sounds, and pulsus paradoxus (a variation in pulse quality associated with respiration).

In the cat, signs also depend on location and extent of tumor. Cats with visceral tumors usually have a history of lethargy, anorexia, vomiting, sudden collapse, dyspnea, or a distended abdomen.[53] On physical examination, pallor, pleural or peritoneal fluid, and a palpable abdominal mass may be detected.

DIAGNOSTIC TECHNIQUES AND WORKUP

Complete staging for an HSA patient typically includes hematology and serum biochemistry, coagulation testing, thoracoabdominal imaging, +/− abdominocentesis and/or echocardiography.

Anemia is often evident in both dogs and cats, usually characterized by the presence of schistocytes (associated with microangiopathic hemolysis) and acanthocytes in the peripheral blood.[12,14,53,56,64] Anemia may be regenerative or nonregenerative depending on chronicity. Blood typing or crossmatching may be indicated if surgery is planned in a severely anemic patient. In addition, a neutrophilic leukocytosis may be seen.[53,56] Thrombocytopenia is observed in 75% to 97% of cases, ranging from mild to severe.[56,65,66] Serum biochemistry changes are typically nonspecific and can include hypoalbuminemia, hypoglobulinemia, and mild elevations in liver enzymes.[56]

A coagulogram is very useful in animals with suspected HSA. The majority of patients show perturbations in some aspect of the coagulation cascade (prothrombin time, activated partial thromboplastin time, platelet count, activated clotting time, fibrinogen concentration, fibrin degradation products) (Table 32-1),[56,65-69] and approximately 50% have coagulation abnormalities meeting the criteria for disseminated intravascular coagulation.[65,67] One study reported a 25% fatality rate from complications associated with coagulopathy in dogs with HSA.[65] Tumor vasculature, especially in the case of HSA, differs significantly from normal vasculature, commonly containing blind-ended or irregular tortuous vessels, incomplete endothelial lining, arteriovenous shunts, exposed subendothelial collagen, and platelet-tumor aggregates.[70]

TABLE 32–1 Coagulation Abnormalities in Dogs with Hemangiosarcoma[56,65-69]

Coagulation Parameter	Percentage Abnormal
Prolonged PT	12.5
Prolonged APTT	46
Thrombocytopenia	75–97
Increased fibrin degradation products	46–93
Hypofibrinogenemia	8–46
Criteria for DIC	47–50

PT: Prothrombin time.

APTT: Activated partial thromboplastin time.

DIC: Disseminated intravascular coagulation.

Figure 32-5

Ultrasound image of canine splenic hemangiosarcoma. *(Image courtesy of R. T. O'Brien.)*

Hemangiosarcoma-derived endothelial cells may also have an "activated" phenotype, further promoting initiation of the coagulation cascade.

HSA effusions are serosanguineous (or frank blood), and usually do not clot. The cytology of effusions is rarely diagnostic—although tumor cells are likely present, they are heavily diluted with peripheral blood.

Screening for metastasis is mandatory prior to definitive surgery for HSA. Dogs with gross evidence of metastasis have a grave prognosis, and surgery is purely palliative. Thoracic radiographs should be obtained in all cases. One study reported a 78% sensitivity and 74% negative predictive value for detecting pulmonary manifestations of HSA, and obtaining three views significantly decreased the false-negative rate.[71] Dogs with hemopericardium secondary to cardiac HSA typically have a globoid cardiac silhouette, with or without distension of the caudal vena cava.[72] Abdominal radiographs may reveal a mid or cranial abdominal mass in many cases; however, abdominal ultrasound is a superior modality for imaging the abdomen. Many animals with visceral HSA will have ascites, which diminishes abdominal detail with radiographs but not ultrasound. Ultrasound also allows for the more thorough evaluation of the rest of the abdomen for evidence of metastatic disease. Hemangiosarcoma typically has a heteroechoic appearance, ranging from anechoic/cavitated to hyperechoic, or a targetoid appearance (Figure 32-5).[73,74] Although ultrasound is sensitive, it is not specific for metastasis.

Dogs with cardiac HSA and hemopericardium exhibit typical electrocardiographic signs consistent with pericardial effusion (decreased amplitude QRS complex, electrical alternans). Echocardiography can be useful in dogs with suspected pericardial effusion, and a right atrial mass may be visible in 65% to 90% of cases (Figure 32-6).[75-78] However, some dogs may develop
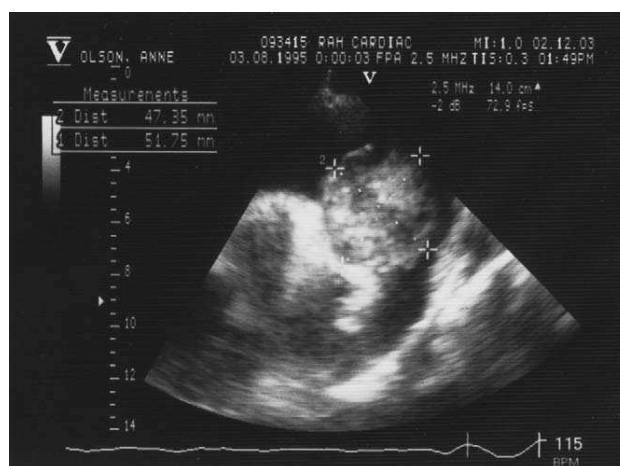
Figure 32-6

Echocardiographic image of right atrial hemangiosarcoma with pericardial effusion. *(Image courtesy of R. A. Henik.)*

blood clots within the pericardium that can have a mass effect. Thus, the absence of a mass does not rule out HSA, and the presence of a mass is not pathognomonic. Despite this caveat, detection of a mass on echocardiography is strongly associated with a worse prognosis in dogs with pericardial effusion.[72,77] Other negative prognostic factors include history of collapse and presence of ascites.[72] Routine evaluation of the heart for metastatic disease in a dog with subcutaneous or visceral HSA without evidence of other metastasis is rarely rewarding. A clinical staging system for HSA is given in Box 32-1.

T primary tumor

T_0 No evidence of tumor
T_1 Tumor less than 5 cm in diameter and confined to primary site
T_2 Tumor 5 cm or greater or ruptured: invading subcutaneous tissues
T_3 Tumor invading adjacent structures, including muscle

N regional lymph nodes

N_0 No regional lymph node involvement
N_1 Regional lymph node involvement
N_2 Distant lymph node involvement

M distant metastasis

M_0 No evidence of distant metastasis
M_1 Distant metastasis

Stages

I T_0 or T_1, N_0, M_0
II T_1 or T_2, N_0 or N_1, M_0
III T_2 or T_3, N_0, N_1 or N_2, M_1

———

Note: 5 cm diameter criteria has not been conclusively shown to impact survival.

Ultimately, a definitive diagnosis of HSA requires a surgical biopsy. Needle aspiration cytology of suspicious masses, while simple and cost-effective, is of low diagnostic utility due to the hemodilution that usually accompanies sampling. Needle core biopsies can be obtained of cutaneous or subcutaneous lesions, but it should not be performed on suspect visceral lesions due to the risk of induction of hemorrhage and subsequent tumor cell seeding of the peritoneum.

Several investigators are evaluating additional tests, which may allow more accurate presurgical diagnosis. Pericardial fluid pH was suggested to correlate with presence or absence of neoplasia in one study;[79] however, other studies have shown pericardial fluid analysis to be of little diagnostic benefit.[80,81] One study evaluated serum concentrations of cardiac troponins and found a significantly higher concentrations of troponin I, an indicator of myocardial damage, in dogs with HSA compared to dogs with idiopathic pericardial effusions.[82] Plasma concentrations of VEGF and urine concentrations of bFGF are elevated in dogs with HSA versus normal controls;[24,25] however, neither was found to correlate with remission status, disease stage, or outcome. Advanced imaging techniques, such as contrast harmonic ultrasound,[83] contrast-enhanced computed tomography,[84] and magnetic resonance imaging[85] have been useful in

discriminating malignant versus benign lesions in preliminary studies. There is evidence that multiparameter flow cytometry of peripheral blood utilizing a variety of endothelial and primitive hematopoietic cell markers may be a promising noninvasive means of confirming an HSA diagnosis.[86]

TREATMENT

Surgery

Surgery remains the primary method of treatment for almost all dogs and cats with HSA. Prior to surgery, appropriate treatment for shock (e.g., crystalloids, colloids) and correction of severe hematologic or coagulation abnormalities should be addressed with blood products as necessary. Surgery should be as aggressive as possible to remove all locally affected tissue. For cutaneous or subcutaneous HSA, surgical considerations are similar to those for other soft-tissue sarcomas (see Chapter 20). For splenic HSA, splenectomy is required. At the time of splenectomy, the abdomen should be thoroughly explored and any suspicious lesions in the liver or omentum should be excised and submitted for histopathology. Biopsy of grossly normal livers is probably not useful. Dogs undergoing splenectomy are prone to develop ventricular arrhythmias following surgery. In one study of 59 dogs, 24% developed arrhythmias.[87] Poor myocardial perfusion secondary to hypoxia, hypovolemia or anemia, or a neurohormonal response associated with manipulation of the spleen are all potential causes. An electrocardiogram should be monitored intraoperatively and in the postoperative period, and arrhythmias should be treated as they arise. Arrhythmias usually resolve within 24 to 48 hours.

Surgery can be performed for primary cardiac HSA. An open or thoracoscopic pericardiectomy can be a palliative procedure, allowing effusion to escape into the thorax rather than accumulating in the pericardium where a small volume can readily restrict function. Right atrial appendage masses can be resected with a stapling device or hand stitching,[88] and reconstructive procedures have been described in cases where extensive resection is required.[89] A retrospective study suggests that dogs undergoing pericardectomy and mass removal that survived the perioperative period have a prognosis similar to dogs with HSA of other visceral sites.[90] Perioperative mortality was 13%.

Chemotherapy

Given the very high metastatic rate of most canine HSA and the poor outcome associated with surgery alone, adjuvant chemotherapy is indicated in all cases, with the exception of pure noninvasive and small cutaneous

tumors. Single-agent and combination doxorubicin (DOX)-based chemotherapy protocols are most frequently used (Box 32-2).[91-96] Other combinations such as vincristine, cyclophosphamide, and methotrexate (VCM) have yielded only modest improvement in survival time.[14] Ifosfamide has been shown to also have some minor antitumor activity in canine HSA as well.[97] In cats, similar DOX-based protocols are employed, although reports of outcome are lacking. For cases where surgery is declined or impossible due to size/location or presence of metastasis, DOX-based chemotherapy induces meaningful tumor regression in only a minority of animals.[98] Remissions for measurable disease are typically incomplete and brief.

Immunotherapy

Few studies have been conducted to evaluate biologic therapy for HSA. One study used a mixed killed bacterial vaccine, following surgery, and showed some improvement in survival time in dogs with splenic HSA.[14] More recently, a surgical adjuvant study was conducted in dogs with splenic HSA to compare chemotherapy (DOX and cyclophosphamide) to the same chemotherapy combined with immunotherapy using liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE). The median survival time for those dogs treated with chemotherapy alone was 5.7 months versus 9.1 months ($p = 0.03$) for those treated with L-MTP-PE and chemotherapy, with 40% of dogs in the L-MTP-PE group experiencing long-term survival.[96] Liposome-MTP-PE is currently unavailable in the United States, but it has been granted orphan drug status for the treatment of pediatric osteosarcoma in the European Union. Investigations of allogeneic tumor cell vaccine approaches in combination with chemotherapy are under way.[99]

Radiotherapy

Radiotherapy (RT) is rarely utilized for HSA due to the anatomic sites involved and high metastatic rate. Coarsely fractionated (palliative) RT for peripheral masses may dramatically decrease local disease but may not significantly impact overall survival.[100,101] It is conceivable that a combination of palliative RT and chemotherapy may provide better control, but this possibility awaits further investigation. Due to their reduced potential for metastasis, full-course (definitive) RT may be a reasonable consideration for dogs with incompletely resected, solitary dermal HSA or feline nonvisceral HSA.

Novel Therapy

Given the endothelial derivation of HSA, therapy directed against angiogenesis is a logical avenue of exploration, and murine HSA has been used as a model for the evaluation of novel antiangiogenic strategies. Antitumor activity has been seen in a murine HSA model to a variety of antiangiogenic treatments, such as VEGFR kinase inhibitors,[102] TNP-470,[103] and several others.[104-106] Interleukin-12 has been shown to retard tumor growth in a canine HSA xenograft as well.[31]

In humans with various blood vascular tumors, antitumor activity has been seen following the local administration of interleukin-2,[107,108] and the local or systemic administration of interferon-alpha in combination with traditional cytotoxics.[109-112] Additionally, pegylated liposomal DOX (Doxil), paclitaxel (Taxol), and docetaxel (Taxotere) appear to have activity against human HSA.[110,113,114]

One study evaluated minocycline, an antibiotic with reported antiangiogenic activity, in combination with surgery and combination chemotherapy (DOX and cyclophosphamide) in dogs with HSA of various stages

Box 32-2	Adjuvant Chemotherapy Protocols for Canine Hemangiosarcoma
1. VAC[a]	
Day 1	Doxorubicin[b] 30 mg/m² IV
	Cyclophosphamide 100 to 150 mg IV
	OR
	Cyclophosphamide 150 to 200 mg PO, divided over 3 to 4 days
Days 8 and 15	Vincristine 0.75 mg/m² IV
Day 22	Repeat cycle for a total of four to six cycles
2. AC[a]	
Day 1	Doxorubicin[b] 30 mg/m² IV
	Cyclophosphamide 100 to 150 mg IV
	OR
	Cyclophosphamide 150 to 200 mg PO, divided over 3 to 4 days
Day 22	Repeat cycle for a total of four to six cycles
3. DOX[a]	Doxorubicin[b] 30 mg/m² IV
	Repeat every 2 to 3 weeks for five treatments

[a]*Perform CBC prior to each chemotherapy treatment – delay treatment for 5 to 7 days if neutrophils <2000/uL or platelets <75,000/uL.*
[b]*Doxorubicin is given at 25 mg/m² or 1 mg/kg in dogs weighing less than 10 kg.*

and locations. No difference in outcome was detected in this single-arm study when compared to historical controls treated with a similar chemotherapy protocol without minocycline.[93] A combination of standard DOX/cyclophosphamide chemotherapy plus inhaled DOX modestly increased survival time and decreased pulmonary metastasis in dogs with splenic HSA, when compared to historical controls,[115] and intracavitary Doxil has been shown to be equivalent to standard systemic DOX-based chemotherapy with respect to survival.[116]

PROGNOSIS

Canine

A summary of the results of several reports on the treatment of splenic HSA is presented in Table 32-2. Overall, the prognosis for dogs with splenic HSA treated by surgery alone is extremely poor; median survival times range from 19 to 86 days, and less than 10% survive to 12 months following surgery alone.[2,8,14,117,118]

Surgery plus DOX-based chemotherapy following surgery increase median survival times to 141 to 179 days.[91,92,94,95] Even with the addition of chemotherapy, the 12-month survival rate is 10% or less. In one study, the addition of immunotherapy (L-MTP-PE) to standard chemotherapy increased median survival time to 273 days.[96] Some studies have shown that stage I (nonruptured splenic tumor) have a more favorable outcome than stage II (ruptured splenic tumor) when chemotherapy is used.[14,93,96] One study employed a histologic grading scheme and demonstrated that dogs with low-grade tumors, albeit rare, had a better prognosis than dogs with intermediate- or high-grade tumors.[92]

In a study of surgically treated cutaneous HSA, tumors involving the dermis (without subdermal invasion) had a median survival time of 780 days (n = 10).[47] Tumors originating or invading into the subcutaneous tissues (n = 10) and muscle (n = 5) had a median survival time of 172 and 307 days, respectively, although there was no statistical difference between subcutaneous and intramuscular locations.[47] Therefore, adjuvant therapy should be offered for subcutaneous or intramuscular HSA. One study reported on the outcome of 76 cats and dogs with "nonvisceral" HSA treated with surgery. More than half of these animals were alive 12 months after diagnosis. Adequacy of surgical excision and dermal versus subcutaneous location were predictors of outcome. Interestingly, more than half of the dogs with subcutaneous HSA survived beyond 1 year. No information was provided regarding usage or type of adjuvant therapy in this study.[11]

Overall, the prognosis is similarly poor for atrial HSA as it is for splenic HSA. In a study of nine dogs undergoing surgery for right atrial HSA, the average survival time was 4 months.[88] In other reports of dogs with HSA involving the pericardium who underwent surgery with or without chemotherapy, median survival ranged from 4 to 7 months.[77,90] Chemotherapy was variably administered in these cases, and there was insufficient statistical power to determine its impact.

Feline

In cats, the prognosis for visceral HSA is poor. Most cats die from recurrence of the primary tumor or metastasis,

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TABLE 32–2 Comparison of Survival Times in Dogs with Splenic Hemangiosarcoma

Treatment	Number of Dogs	Median Survival Time (Days)	References
Splenectomy	Various	19–86	2,8,14,116,117
Splenectomy + MBV	10	91	14
Splenectomy + MBV + VMC	10	117	14
Splenectomy + Ifosfamide	6	142	96
Splenectomy + VAC	6	145	90
Splenectomy + AC	6	179	94
Splenectomy + AC	16	141	95
Splenectomy + AC + L-MTP-PE	16	273	95

MBV = Mixed bacterial vaccine.

VMC = Vincristine, methotrexate, cyclophosphamide.

VAC = Vincristine, doxorubicin, cyclophosphamide.

AC = Doxorubicin, cyclophosphamide.

L-MTP-PE = Liposome muramyl tripeptide phosphatidylethanolamine.

and in five cats undergoing splenectomy, the median survival time was 20 weeks.[53] Hemangiosarcomas located in cutaneous and subcutaneous sites have recurrence rates of 60% to 80%.[53,58] In one study of 10 cats undergoing "complete" excision of cutaneous HSA, recurrence occurred in cats on an average of 4 months.[53] Metastasis can develop following surgical resection in some cases, although the frequency is unknown.[63]

In summary, surgery still offers the best approach to diagnose and treat HSA even though it is generally only palliative. The effectiveness of adjuvant chemotherapy needs further evaluation. New approaches to treatment, such as immunotherapy or antiangiogenic therapy, may provide alternatives, and additional investigation into the biology of canine HSA, which researchers hope will lead to clinical trials, is desperately needed.

COMPARATIVE ASPECTS

A spectrum of endothelial tumors—including hemangioma, hemangioblastoma, Kaposi's sarcoma, and angiosarcoma—is seen in humans. Angiosarcoma is extremely rare in humans and can be associated with breast conserving radiotherapy in women treated for breast cancer.[119] With this exception, it has a lesion distribution and behavior similar to canine HSA. As in dogs, metastasis is frequent and adjuvant chemotherapy provides only minimal benefit.

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SECTION B
Thymoma

Stephen J. Withrow

INCIDENCE AND RISK FACTORS

Thymomas are rare in the dog and even less common in the cat. Even though the normal thymus is larger and more active in puppies and kittens, the disease is generally diagnosed in older animals (dogs—9 years; cats—10 years). No consistent breed predilection is known; however, medium- and large-breed dogs may be overrepresented.[1,2] One study demonstrated a female prevalence,[3] although most studies show no sex predilection.[1]

PATHOLOGY AND NATURAL BEHAVIOR

Thymomas originate from thymic epithelium but are variably and even predominantly infiltrated with mature lymphocytes. The epithelium is the neoplastic component. Squamous cell carcinoma has been rarely documented to arise within feline thymomas,[4] and thymolipoma has been reported in a cat.[5] Different histologic cell types can be seen (differentiated epithelial, lymphocyte rich, and clear cell), but prognostic differences between these groups are not apparent.[6] Approximately 60% of feline thymomas are cystic and the metastatic rate is reported to be 20%.[7] "Benign" thymomas are noninvasive and well encapsulated, while "malignant" thymomas invade adjacent structures (precava, rib cage, and pericardium) but rarely metastasize.[6,8] The terms *benign* and *malignant* are derived more from their clinical features (resectability) than from histologic features. Large lesions do not necessarily imply nonresectability. Distant metastasis is rare but has been reported.[9]

The most common anterior mediastinal tumors are lymphomas and thymomas followed by branchial cysts, ectopic thyroid, chemodectoma, and a variety of rare neoplasms.[1,10-12] Sarcomas from the rib or sternum with intrathoracic extension may present similarly to a primary mediastinal mass.

HISTORY AND CLINICAL SIGNS

Most patients present with signs of respiratory distress (coughing, tachypnea, and dyspnea). Precaval syndrome (facial, neck, or front leg swelling) may also occur secondary to obstruction of venous and lymphatic drainage from the head, neck, and legs. The paraneoplastic syndrome of myasthenia gravis has also been demonstrated in up to 40% of dogs and the rare cat and is characterized by muscle weakness and megaesophagus.[1-3,13-18] As many as 40% of dogs will present with megaesophagus or aspiration pneumonia.[6,17] In 20% to 40% of patients, nonthymic neoplasms, various autoimmune diseases such as immune-mediated anemia, and polymyositis have also been associated with the presence of thymoma.[2,3,17,19] Exfoliative dermatitis has been associated with thymomas in cats (Figure 32-7).[20-22]

DIAGNOSTIC TECHNIQUES AND WORKUP

Physical exam findings may include painless, bilateral pitting edema of the head, neck, or front legs as a result of precaval syndrome. Jugular veins may be enlarged and tortuous. Auscultation of the thoracic cavity may reveal decreased lung sounds over the anterior mediastinum (mass) or ventral lung fields (pleural effusion). The heart sounds may be heard more dorsal and caudal than normal as a result of cardiac displacement. In smaller dogs and cats, decreased compressibility of the anterior thoracic cavity may also be detected.

Figure 32-7

Exfoliative dermatopathy in a cat with a thymoma. Dry exfoliative scurf is observed lifting off skin surface throughout the hair coat. *(Photograph courtesy of Dr. Nick Bacon, Colorado State University.)*

Figure 32-8

Anterior mediastinal mass is seen on lateral radiograph of a 13-year-old female mixed-breed dog.

Figure 32-9

Cross-section of well-encapsulated thymoma surgically removed from the patient in Figure 32-8. Note the cystic nature of lesion that may complicate cytologic diagnosis. The patient is alive and free of disease over 2 years postoperatively.

Routine hematologic and biochemistry tests are usually normal, although lymphocytosis has been reported.[6,9,23] Hypercalcemia is only rarely reported with thymomas but will confuse the diagnosis with thymic lymphoma.[6,24-26] Thoracic radiographs generally reveal a variably sized mass in the anterior mediastinum, pleural effusion, and displacement of the cardiac silhouette caudally and dorsally (Figure 32-8). Dilatation of the esophagus may be seen with myasthenia gravis.

Transthoracic fine-needle aspiration for cytologic preparations is a simple and safe procedure, but it has been associated with unreliable results in the author's experience. Cytologic results usually reveal a preponderance of lymphocytes rather than the epithelial component of the actual tumor. Mast cells are also common.[6] Thymomas are commonly cystic (Figure 32-9) and frequently yield nondiagnostic material.[2,7] Since the major differential diagnosis is lymphoma, which is treated differently, this distinction is important.[27] Flow cytometry of the thymic mass may help differentiate thymic lymphocytes from thymic lymphoblasts (lymphoma).[28] As opposed to thymoma, feline thymic lymphoma is usually seen in young cats (with a mean age of 2 years) that are feline leukemia virus positive (80%)[29] (see Chapter 31, Section B). Canine thymic lymphoma is often associated with hypercalcemia (25% to 50%) or generalized lymphadenopathy.[30] Cytologic evaluation of pleural effusion or the mass in thymoma cases should yield mature lymphocytes as opposed to lymphoblasts seen with lymphoma. Transthoracic needle core biopsy can be diagnostic but will often yield cystic and necrotic material with a preponderance of lymphocytes, making definitive diagnosis difficult.
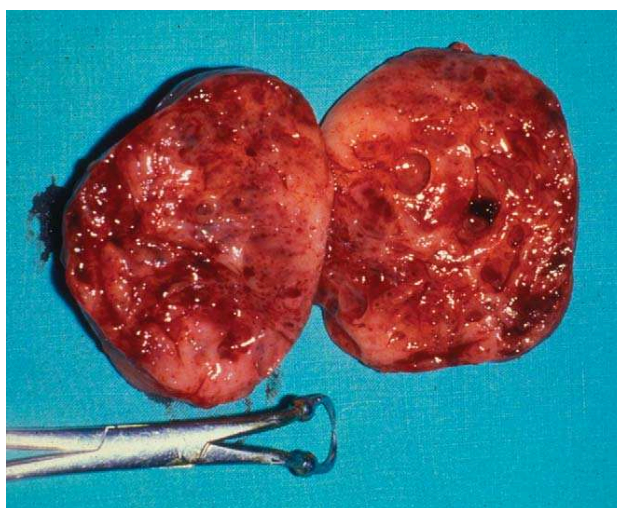
Angiography, ultrasound, and computed tomographic evaluation of mediastinal masses should be further evaluated for their sensitivity in differentiating thymoma from lymphoma.[31-33] Computed tomography of 14 dog and cat mediastinal masses showed no correlation to final histology although it did help with local staging.[34] Ultrasonography of thymomas generally suggests a pattern of mixed echogenicity with cavitation,

whereas lymphoma more commonly has a homogeneous hypoechoic mass.[32]

The definitive presurgical diagnosis of thymoma is difficult and frequently rests on trying to rule out the more common diseases. Most thymic lymphoma cases respond rapidly and completely to aggressive chemotherapy. Thymoma should be suspected in patients with a partial remission or stable disease 10 to 14 days after chemotherapy administration designed for lymphoma.

THERAPY

The definitive therapy for thymoma is surgical resection. Smaller masses may be approached via an intercostal thoracotomy, but the more common large masses should be approached via a sternotomy (Figure 32-10). Once the mass is seen, the surgeon must make a clinical judgment as to resectability. Approximately 70% of thymomas are resectable, and nothing (including size) is uniformly predictive in the preoperative evaluation. Invasive and malignant thymomas will be adherent to surrounding tissues, especially major nerves, veins, trachea, pericardium, and esophagus, making removal difficult. Resection of thymomas, including precaval thrombi, can be performed.[33]

Debulking of invasive thymomas can be attempted in hopes of alleviating symptoms of the physical mass or possibly enhancing potential chemotherapy or radiation treatment but may be associated with extensive morbidity. If the mass is deemed unresectable, and if other treatments are to be pursued, large wedge biopsies should be taken at surgery or thoracoscopy, keeping in mind that thymomas are not homogeneous and are often cystic.

Attempts at treatment with chemotherapy, similar to those used to treat lymphoma, have been reported, but objective partial or complete remissions are less common than for lymphoma.[2,6,35,36] Chemotherapy has gained in popularity in treating humans with thymoma and should be evaluated more in animals, especially those with megaesophagus, who are poor surgical risks.[37-39] Radiation therapy has rarely been attempted in animals, but either chemotherapy or radiation should theoretically decrease the lymphoid component of the mass.[40,41] Seventeen dogs and 7 cats received radiation for thymomas. Complete responses were noted in 20%. The median survival for dogs was 248 days and cats 720 days. The role of radiation was not clearly elucidated since many animals received concomitant surgery or chemotherapy.[42]

Myasthenia gravis, if present, generally requires treatment with immunosuppression or anticholinesterase drugs but may not be reversible.[1,6,14,17,46,47] Symptomatic treatment of the megaesophagus (motility drugs, H_2 blockers, antibiotics) may be attempted in hopes of decreasing the prevalence of regurgitation and aspiration pneumonia.[17,43]
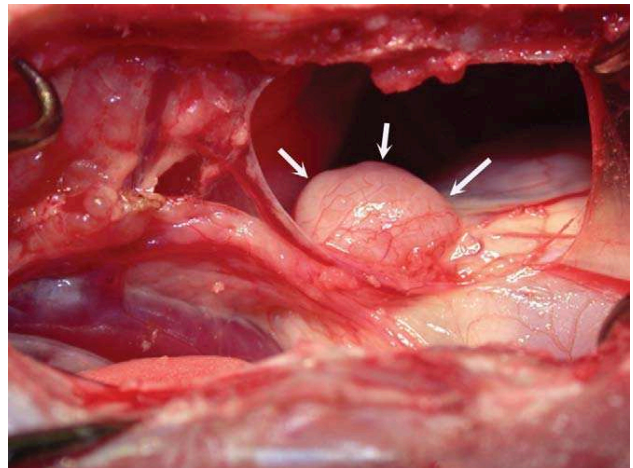
Figure 32-10

Presence of a discrete encapsulated thymoma *(arrows)* attached to the pericardium in a cat observed during a median sternotomy. *(Photograph courtesy of Dr. Nick Bacon, Colorado State University.)*

PROGNOSIS

The prognosis for surgically resectable (benign) thymomas in dogs without megaesophagus is good. Long-term remissions and cures can be expected in dogs without megaesophagus or aspiration pneumonia with resectable tumors.[6,9,13,35,44,45] Dogs *without* megaesophagus and a resectable tumor had an 83% 1-year survival (Figures 32-11 and 32-12).[6]

The outlook for patients with nonresectable thymomas remains poor, although chemotherapy or radiation therapy may be beneficial[42] and in a few instances has made a previously unresectable thymoma resectable.

Myasthenia gravis may or may not improve with complete removal of the thymoma, and improvement may require months.[1,6,15,17,46,47] Two dogs with untreated thymoma followed with thoracic radiographs lived 6 and 36 months, implying slow growth of some thymomas.[3]

Surgical resection of thymomas in 12 cats suggests a good prognosis. Two cats died in the perioperative period, while none of the remaining 10 cats developed local recurrence or metastasis. The median survival was almost 2 years. Myasthenia gravis developed postoperatively in 2 cats.[48] Cystic thymomas in cats carry a good prognosis.[7]

COMPARATIVE ASPECTS[49]

Thymomas are very similar in animals and humans. Sixty-five percent (65%) are encapsulated and noninvasive, while 35% are invasive.
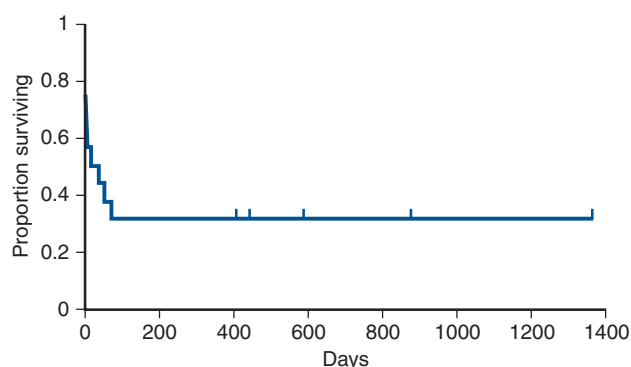
Figure 32-11

Kaplan-Meier survival curve for 23 dogs with thymoma.
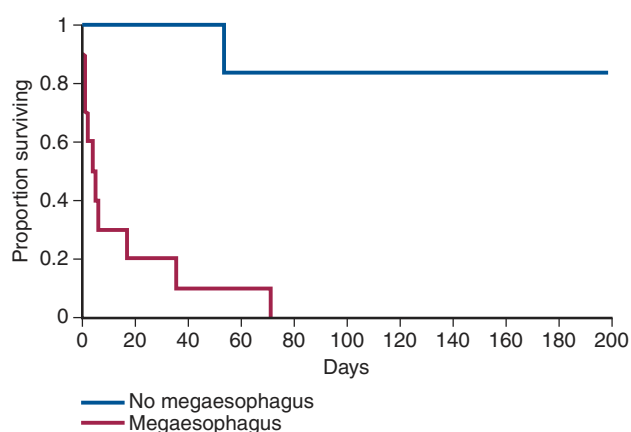
Figure 32-12

Kaplan-Meier survival curves for 23 dogs with thymoma, 11 of which had megaesophagus and 12 of which did not have megaesophagus.

Multiple paraneoplastic syndromes have been associated with thymomas (autoimmune, endocrine, infectious, and nonthymic cancer). Up to 50% of patients will have symptoms of myasthenia gravis. Muscle strength will improve in only 25% of these patients after removal of the thymoma.

Treatment is surgical removal with at least 80% free of disease at 5 years. Radiation therapy is indicated for invasive thymomas, and they are considered moderately sensitive.[50] Corticosteroids have caused regressions of some thymomas. Other single agents with some efficacy include doxorubicin, cisplatin, and various alkylating agents.[51]

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SECTION C
Canine Transmissible Venereal Tumor

Louis-Philippe de Lorimier and Timothy M. Fan

INCIDENCE AND RISK FACTORS

Canine transmissible venereal tumor (TVT), also known as transmissible venereal sarcoma and Sticker's sarcoma, is a naturally occurring horizontally transmitted tumor of dogs that is occasionally observed in other canids (foxes, jackals, coyotes).[1-5] It has historically been given other names, such as venereal granuloma, canine condyloma, contagious lymphoma, or infectious sarcoma.[1,3] While TVT has a worldwide distribution, the prevalence is higher in temperate climates, and enzootic areas include the southern United States, Central and South America, certain parts of Africa, the Far East, the Middle East, and southeastern Europe.[1-6] In some regions, where breeding is poorly controlled and free-roaming dogs are found in high concentrations (urban areas), TVT is by far the most common canine tumor.[1-3,6] Both increased rainfall and mean annual temperature are positively correlated with prevalence.[6-8] Occasional cases have been known to occur in regions otherwise free of TVT, following travel to an endemic area.[9]

Coitus being the classic mode of transmission, free-roaming sexually intact mature dogs of either gender are at highest risk to develop TVT. There appears to be no breed overrepresentation, and a high percentage of dogs reported in published studies are mixed breeds.[1-8] The most common primary sites of involvement are the external genitalia, but other sites can be affected on occasion, including the nasal and oral cavities, subcutaneous tissues, and the eyes.[1-4, 10-13] Spontaneous regressions are observed with canine TVTs, both experimentally and naturally acquired, and many studies have shown various immune responses to be operative at different tumor stages.[4,6,8,14-22] It therefore seems rational that immunosuppression of any nature could be a risk factor to acquire and maintain TVTs and may lead to a higher potential for dissemination.

TVT is transmitted directly from dog to dog, across major histocompatibility complex (MHC) barriers and through transplantation of viable tumor cells on

damaged mucosal surfaces, during sexual intercourse or other social behaviors such as sniffing and licking.[1-8] This characteristic of TVT is unique, in that it is a naturally occurring tumor transmitted as an allograft, which behaves more or less like a parasite that becomes autonomous from the original host for continued growth and survival.[4] A viral etiology has been suspected, and though some authors have described C-type particles and others have claimed transmission possible with cell-free extracts, viral particles have not been observed via electron microscopy.[5,23-25]

PATHOLOGY AND NATURAL BEHAVIOR

Interestingly, TVTs of dogs from all parts of the world appear to have a relatively constant karyotype of 59 chromosomes (reported range 57-64).[4,25-28] This also appears consistent in metastatic lesions, or after multiple *in vivo* or *in vitro* passages and is markedly different from the normal canine karyotype of 78 chromosomes.[29,30] Of the 59 chromosomes, 13 to 17 are metacentric and 42 to 46 are acrocentric.[25,26] These specific and consistent anomalies support a cellular mode of transmission.

Another distinctive and consistent alteration observed in TVTs from different geographical areas, further supporting a cellular mechanism of transmission, is that of transposable genetic elements at the DNA level. Canine TVTs from various parts of the world all have been shown to have so-called long interspersed nuclear elements (LINE) upstream of the region coding for the oncogene c-*myc*.[20,31-37] This specific molecular alteration whereby LINE, in a retroviral-like fashion, is inserted upstream of c-*myc* possibly leading to amplified gene transcription, is believed to be a player in the tumorigenesis of canine TVT.[35] In addition, the identification of the TVT-specific LINE insertion can serve as a useful molecular tool, both to assess the completeness of remission and evaluate minimal residual disease or to differentiate other tumors from the occasional TVT that may be morphologically atypical.[33,36,37] A point mutation in the tumor suppressor gene *p53* was demonstrated in TVT tissue samples.[35] Mutation of such a key regulator of various cellular functions may well be another important molecular event in the oncogenesis of TVT and warrants further investigation.

Commonly recognized and described as a round (or discrete) cell tumor, TVT is suspected to be of histiocytic origin. This is supported by positive reactions to lysozyme, alpha-1-antitrypsin, vimentin, and a macrophage-specific immunostain (ACM1), and negative staining with immunostains specific for other cell types.[4,38-40] Immunohistochemistry has been helpful in confirming metastatic TVT of various anatomical locations.[41-43] Furthermore, two reports described TVT cells

with intracellular *Leishmania* organisms, also suggesting a histiocytic origin.[13,44]

As previously mentioned, the immune system appears to play an important role in the natural progression of TVT in dogs. Canine TVTs transition through phases of growth and progression (P) initially, followed by stasis, and either spontaneous regression (R) in immunocompetent animals, or dissemination in immunosuppressed dogs and inoculated puppies.[8,14-22,45-48] This demonstrates that TVT is antigenic and was the basis for numerous immunologic studies. A tumor associated antigen has been demonstrated, and circulating levels appear to correlate positively with tumor volume, with disappearance 48 to 72 hours after surgical removal.[2,4,8,45,49] Canine TVT cells in P phase apparently produce a molecule capable of causing apoptosis of circulating B lymphocytes, favoring evasion of immune surveillance through decreased production of host antibodies.[21] Studies show that TVTs in R phase contain more infiltrating T lymphocytes (TIL) and that tumor cells demonstrate an increased expression in MHC class I and II.[4,15-17,46] Increased MHC molecule expression appears to be stimulated by the TILs in the R phase.[16] Production of transforming growth factor-1 (TGF-1) by P phase TVT cells may inhibit lymphokine-activated killer (LAK) activity. However, increased interleukin-6 (IL-6) production by TILs in R phase antagonizes the effects of TGF-1 and restores LAK activity.[22] Circulating lymphocytes from dogs in postregression phase have inhibitory or cytotoxic effects on TVT cells, contrary to lymphocytes of normal dogs or TVT-bearing dogs in P phase.[17,50] Passive transfer of postregression sera to TVT-bearing dogs has been shown to inhibit and prevent tumor development when administered simultaneously with transplantation, emphasizing the importance of antibody-dependent cellular cytotoxicity.[8,18,19,51]

The latency period for tumor development may be in the range of 2 to 6 months.[2,9] Many naturally acquired TVTs may undergo spontaneous remission, doing so within 3 months following implantation, but regression without therapy is unlikely if the tumor has been present for 6 months or longer.[2] When present in immunodeficient or young puppies, TVTs can metastasize and are unlikely to spontaneously regress.[14,29,45] Numerous reports describe metastatic TVTs, and the incidence of dissemination at diagnosis can be as high as 17%.[10] Reported primary and metastatic sites include the regional lymph nodes, skin, brain, eyes, bone, kidney, and orbit.[2,10,41,42]

HISTORY AND CLINICAL SIGNS

The archetypical patient with TVT is a sexually intact young adult dog, living in an endemic area or having traveled to such a place, with a history of social contacts with dogs of similar signalment. The external genitalia

Figure 32-13

Typical appearance of a TVT in a male dog. The tumor is located at the base of the penis, has a cauliflower-like appearance, is very friable, and bleeds easily.

Figure 32-14

Male dog with cutaneous dissemination of TVT on the ventral abdomen. *(Photo courtesy of Pr. Noeme S. Rocha.)*

Figure 32-15

Mucocutaneous TVT of the anal area in a dog. *(Photo courtesy of Pr. Noeme S. Rocha.)*
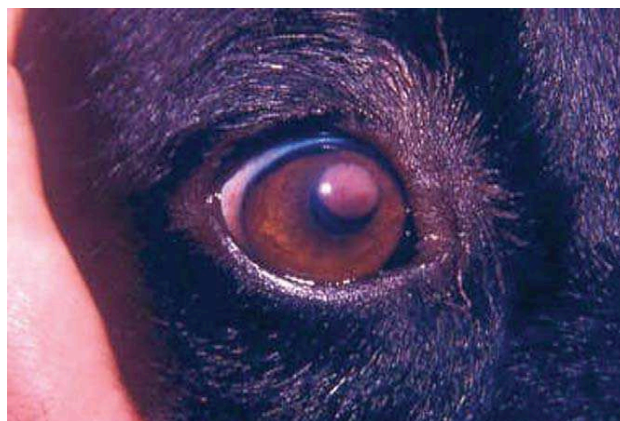
Figure 32-16

Corneal involvement with TVT in a dog. *(Photo courtesy of Pr. Noeme S. Rocha.)*

are the most common primary location of naturally acquired TVTs. Affected dogs often manifest with chronic signs of discomfort or bloody discharge from the vulva or penile sheath for weeks to months prior to diagnosis. In female dogs, the tumor is usually located caudally in the vagina or in the vestibule. In male dogs, the classic location is at the base of the glans penis, requiring caudal retraction of the penile sheath for visual identification (Figure 32-13). The characteristic appearance of TVTs is that of a cauliflower-like friable and vascularized mass. A common clinical sign, independent of the primary location, is a hemorrhagic or serosanguineous discharge. Dogs with primary nasal TVTs most often present for epistaxis and sneezing and occasionally for facial deformity.[1,3,11]

Other occasionally reported primary sites include the skin and subcutis, oral cavity, the eyes, and the anus (Figures 32-14 through 32-16).[1-4, 10-13] Dogs with the classical location of TVT appear to be at higher risk to develop ascending urinary tract infections.[52]

DIAGNOSTIC TECHNIQUES AND WORKUP

Geographical location, signalment, history, clinical signs, and physical examination may provide a presumptive diagnosis of TVT in dogs with the classic presentation. Definitive diagnosis is obtained with cytopathologic or histopathologic analysis of the tumor cells and tissues. Often described as a discrete (or round) cell tumor, TVT

has a characteristic morphologic appearance on cytopathology and is most often readily diagnosed with confidence by this modality, without the need for histopathology (Figure 32-17). Exfoliative cytology demonstrates discrete cells that are round to oval, with moderately abundant pale blue cytoplasm, an eccentrically located nucleus, with occasional binucleation and mitotic figures. Single or multiple nucleoli are often observed, surrounded by clumped chromatin. The most characteristic feature of TVT cells is the presence of numerous discrete clear cytoplasmic vacuoles. Histopathology may be used to confirm the diagnosis, and as previously mentioned, immunohistochemistry has been helpful to diagnose TVT involving unusual metastatic sites.[41-43] Additionally, powerful and specific molecular techniques may be useful for the diagnosis of atypical TVTs or to evaluate minimal residual disease.[33,36,37] Other round cell tumors, including lymphomas, mast cell tumors, plasma cell tumors, histiocytoma, and some melanomas, are important differential diagnoses but are generally not confused with TVT on cytopathology. When diagnosed in regression phase, TVTs contain a higher number of infiltrating lymphocytes.[4,15-17,46]

The incidence of disseminated disease is lower than 15% in most studies. Nonetheless, evaluating regional lymph nodes for metastasis, along with a palpation and cytopathologic evaluation, is always recommended. A thorough physical examination is essential to rule out other possible sites of primary involvement, such as the nasal and oral cavities, the eye and orbit, and the skin and subcutis. Diagnostic imaging is rarely required, with the exception of the occasional invasive TVTs of the nasal cavity, orbit, or other unusual locations. Complete blood cell count, serum biochemistry profile, and urinalysis do not reveal specific changes. A paraneoplastic erythrocytosis has been reported in a few dogs with large tumor burdens and may require temporary symptomatic therapy.[8]
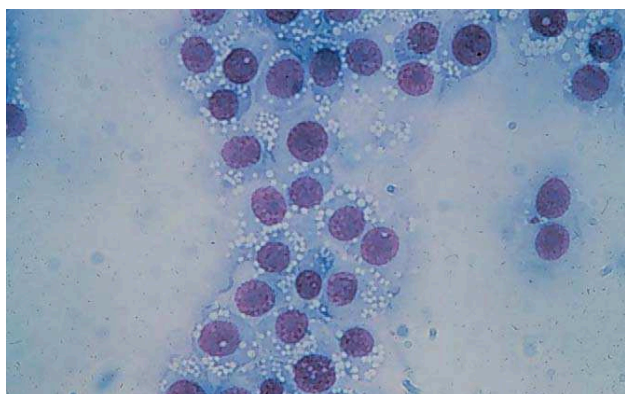
THERAPY

While many therapeutic modalities have shown efficacy against canine TVT, the best described and most effective approach remains monotherapy with the tubulin binding agent vincristine. Vincristine is relatively safe, inexpensive, and provides a complete and durable response in over 90% to 95% of treated dogs, typically following two to six weekly treatments.[1,2,10-12,46,53-55] Other chemotherapy agents or combination protocols incorporating vincristine have been investigated, but none offers a clear advantage over vincristine monotherapy.[54-56] Vincristine is generally administered at a dosage of 0.5 to 0.75 mg/m^2 IV once weekly for three to six treatments. The anthracycline doxorubicin, at 25 to 30 mg/m^2 IV every 21 days for two to three cycles, has been useful for TVT cases demonstrating resistance to vincristine therapy.[10,12,53,57]

Radiation therapy has also demonstrated efficacy in treating TVT. In a study using orthovoltage radiation, all of 18 dogs treated responded with a complete and durable remission.[58] In 7 of these dogs, the solitary TVTs were cured with a single coarse fraction of 1000 cGy. The 11 other dogs required 2 or 3 fractions to achieve a complete response. In another study using megavoltage radiation delivered by a Cobalt-60 unit, 15 dogs treated with radiation alone achieved complete and durable responses with three fractions administered over 1 week, for an average minimum dose of 1500 cGy.[10] The use of radiation therapy to treat canine TVT is supported by these studies, and could be considered a valuable alternative to chemotherapy for lesions showing resistance to systemic agents or located in sanctuary sites (brain, testicles, eye).

Surgery has been reported for solitary or metastatic lesions and can be effective in select cases. However, with an overall recurrence rate around 30% to 75%, and in light of the superior efficacy of other therapeutic modalities, surgical excision is a less attractive treatment alternative.[59,60]

Other sporadically described therapies include biologic response modifiers, piroxicam, cryosurgery, and radiofrequency ablation in a research setting.[1,2,57,61,62]

PROGNOSIS

Given that a number of immunocompetent dogs may experience spontaneous regression and that a vast majority of dogs treated with single-agent vincristine or conservative radiation protocols will obtain complete and durable clinical remissions, the overall prognosis of canine TVT is generally considered very good to excellent.

Figure 32-17

Cytopathologic sample of a canine TVT. Note the discrete round cells, somewhat eccentric nuclei, moderate amount of cytoplasm, and characteristic multiple discrete clear cytoplasmic vacuoles.

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SECTION D
Mesothelioma

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INCIDENCE AND RISK FACTORS

Mesothelioma is a rare neoplasm of dogs and cats affecting the cells lining the coelomic cavities of the body. In 1962, Gerb et al. cited reports of one case of mesothelioma in 1000 dogs and three cases in 5315 dogs.[1] In dogs, primary mesothelial tumors affecting the thoracic cavity, abdominal cavity, pericardial sac, and vaginal tunics of the scrotum have been reported.[2-4] In the cat, primary mesotheliomas have been reported in the pericardium, pleura, and peritoneum, as well as throughout the abdomen with lung and mediastinal lymph node metastases.[5-9] Exposure to asbestos may be an important contributory factor to mesothelioma development in pet dog populations. Affected dogs often have owners who have jobs or hobbies for which exposure to asbestos is a known risk.[10] The level of asbestos in lung tissues of affected dogs has been documented to be greater than controls.[10,11] Asbestos refers to a family of silicate minerals that crystallize into long, flexible fibers. The fibers are categorized into two groups: thin rodlike amphiboles and long curly serpentine, the main type being chrysotile. In humans, much greater risk has been related to amphibole asbestos compared to chrysotile exposure.[12] Chrysotile accounts for 90% of asbestos used worldwide.[12]

The underlying mechanisms of the neoplastic transformation of mesothelial cells, despite its association with asbestos, is not completely understood. Asbestos interacts with mesothelial cells via direct and indirect mechanisms and is associated with both phenotypic and genetic changes in the affected cells. Chromosomal missegregation, aneuploidy, and deletions are reported.[12,13] Loss of tumor suppressor gene products is thought to contribute to the transformation of the mesothelial cells.[13] Also, reactive oxygen and nitrogen species generated by macrophages as the cellular response to asbestos fiber phagocytosis and by the fibers themselves adds to the genetic damage in the tumor precursor cells.[12] Numerous growth factors (e.g., insulin-like growth factor-1 [IGF-1], platelet-derived

growth factor [PDGF], and vascular endothelial growth factor [VEGF]) produced by stimulated macrophages or mesothelial cells are likely important in the pathogenesis of mesothelioma.[13,14] A report of five golden retrievers that developed pericardial mesothelioma after a long-term (30 to 54 months) history of idiopathic hemorrhagic pericardial effusion (IHPE) supports the concept that chronic inflammation may lead to neoplastic transformation in canine mesothelial cells.[15]

Mesothelial tumors occur most often in older animals; however, in cattle and sheep, newborn or young animals are affected.[16] Juvenile mesothelioma was reported in an 11-month-old mixed-breed dog; no underlying etiology was discovered.[17] A report of a 7-week-old puppy with mesothelioma suggests a congenital form may exist.[18]

PATHOLOGY AND NATURAL BEHAVIOR

The normal mesothelium is a monolayer of flattened mesothelial cells. These cells are distinguished by the presence of microvilli, desmosomes, and evidence of phagocytic potential. Disease conditions associated with inflammation or irritation of the lining of the body cavity commonly result in a marked physiologic proliferation of the mesothelial cells. Fluid accumulation in a body cavity promotes exfoliation and implantation of mesothelial cells. Mesotheliomas are considered malignant due to their ability to seed the body cavity, resulting in multiple tumor growths. Distant metastasis is rare.

Mesothelial cells appear morphologically as epithelial cells; however, their derivation is from mesoderm. Mesothelioma can appear histologically as epithelial, mesenchymal, or biphasic, a combination of the two.[19] The epithelial form, which resembles carcinoma or adenocarcinoma, is by far the most common form in small animals. There are also several reports of a variation of the mesenchymal form, which resembles sarcoma and is referred to as sclerosing mesothelioma.[3,20,21] The biphasic form of mesothelioma has been reported in two dogs.[22,23] A cystic peritoneal mesothelioma has also been reported in the dog. This is a rare, benign, slowly progressive form of mesothelioma in humans, which is treated with surgical excision when the disease is localized.[24]

HISTORY AND SIGNS

Classical mesotheliomas occur as a diffuse nodular mass or multifocal masses covering the surfaces of the body cavity (Figure 32-18). Extensive effusions occur due to exudation from the tumor surface or from tumor-obstructed lymphatics; therefore, the most common presenting sign is dyspnea from pleural effusion or a distended abdomen from peritoneal effusion. Dogs with pericardial mesotheliomas can present with acute tamponade and right-sided heart failure.[25,26]

Sclerosing mesothelioma is a variation of mesothelial tumor seen primarily in male dogs, and German shepherds are overrepresented.[3,20] These tumors present as thick fibrous linings in the abdominal or pleural cavities (Figure 32-19). Restriction occurs around organs in the affected area, and in the abdomen such changes can impinge on organs and lead to vomiting and urinary problems.
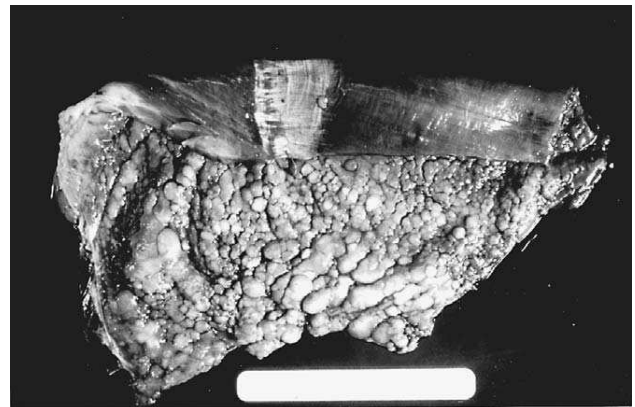
Figure 32-18

The diaphragm from a dog with abdominal mesothelioma is folded to show the affected side and the unaffected side.

Figure 32-19

Loops of intestine from a dog with sclerosing mesothelioma. The thickened serosal surfaces must be distinguished from chronic inflammation.

DIAGNOSTIC TECHNIQUES AND WORKUP

Mesothelioma should be suspected in adult dogs presenting with a history of chronic, nonspecific disease and fluid accumulation in any of the body cavities. Routine echocardiography and abdominal ultrasound are not typically helpful as the tumor cells cling to epithelial surfaces and a mass lesion is rarely noted.[27] Thoracic CT may be of benefit in identifying nodular lesions and assessing lung parenchyma in the face of pleural effusion (Figure 32-20).

Cytologic evaluation of fluid can be diagnostic for other disease processes, such as infection or lymphoma, but will not conclusively diagnose mesothelioma. Mesothelial cells proliferate under any circumstance associated with fluid accumulation in the body cavity, making the distinction between physiologic mesothelial proliferation and neoplasia difficult. Although malignant mesothelial cells easily exfoliate into effusion fluid, they are hard to distinguish from reactive hypertrophic mesothelial cells cytologically. Reactive mesothelial cells display many cytologic features of malignancy, making a definitive diagnosis of neoplasia via cytology impossible in most cases. Although one study found pericardial fluid pH analysis to be a discriminatory test to differentiate benign from malignant effusions, a subsequent study found too much overlap in the pH values for the test to be of benefit.[28,29] Fibronectin concentrations have also been evaluated in pleural effusions in dogs and cats and were found to differentiate malignant or inflammatory causes from cardiogenic effusions. Elevations in fibronectin were a very sensitive but nonspecific test for malignant effusions. Mesothelioma could be ruled out if fibronectin levels were not increased.[30]

Establishing a definitive diagnosis of malignant mesothelioma may be difficult, particularly early in the disease. The diagnosis of mesothelioma requires adequate tissue sampling, preferably from an open, visually directed biopsy. The increasing availability of thoracoscopy and laparoscopy for small animals provides a less invasive way to evaluate these cases.[31] In either procedure, the clinician is encouraged to biopsy any body cavity lining when an obvious cause for fluid accumulation is not found. Sclerosing mesothelioma must be distinguished from chronic inflammatory diseases of the body cavity, such as chronic peritonitis, and histological examination of biopsy material is essential to establish the diagnosis. Additionally, the presence of embolized, non-neoplastic mesothelial cells within lymph nodes is a rare finding in humans with cavity effusions and has been reported in dogs affected with idiopathic hemorrhagic pericardial effusion; therefore, care must be taken so as
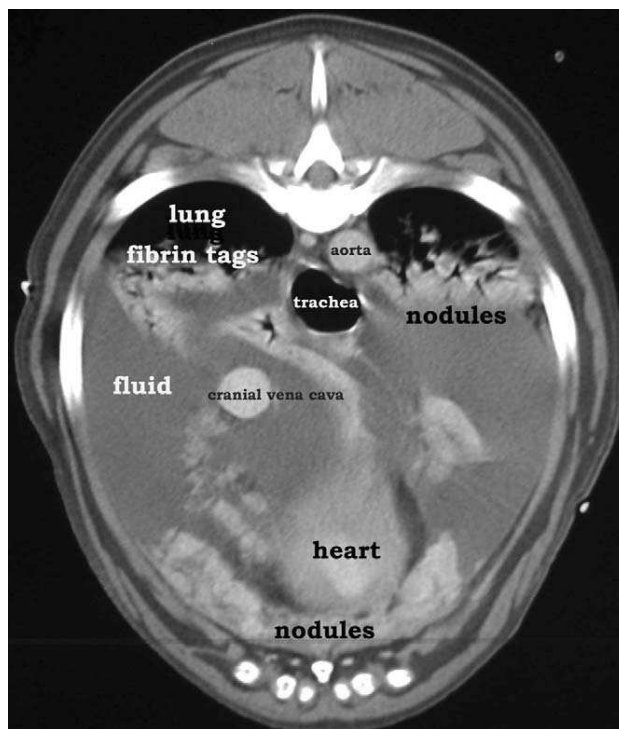
Figure 32-20

A thoracic CT (with contrast) from a dog with histologically diagnosed mesothelioma. The effusion resolved following the first of five doxorubicin chemotherapy (30 mg/m^2, q3wk, IV) treatments.

not to overinterpret these cells as indicative of a metastatic process.[32]

The most useful criteria in establishing a diagnosis of mesothelioma is to demonstrate that the tumor is primarily a neoplasm of the coelomic cavity lining and that the principle method of tumor spread is by transcoelomic implantation. Mesothelioma should be considered when the bulk of the neoplastic tissue exists on the coelomic surface. Histologically, mesotheliomas need to be differentiated from carcinomas, adenocarcinomas, or sarcomas, depending on the morphologic type of the mesothelioma. Unfortunately, there are no cellular markers that conclusively define the mesothelial cell. Advances in immunohistochemical staining have provided additional ways to examine neoplastic cells to help differentiate mesothelioma from other epithelial or mesenchymal tumors in humans. Cytokeratin expression is often positive in mesotheliomas and may help differentiate sarcomatous mesotheliomas from sarcomas.[19] Differentiation of malignant epithelial mesotheliomas from adenocarcinomas can be aided by application of a panel of

different immunohistochemical stains, including calretinin, which stains most mesotheliomas but not adenocarcinomas.[19,21]

TREATMENT AND PROGNOSIS

No satisfactory treatment exists for mesothelioma. Radical excision may benefit some animals, but usually the tumors are too advanced locally and have spread by implantation early in the course of disease. Pericardiectomy may palliate mesothelioma patients that present with cardiac tamponade; two dogs treated with surgery alone survived 4 and 9 months in one study.[33] In another report, the median survival in five dogs treated with pericardiectomy was 13.6 months; three of these dogs received adjuvant intravenous chemotherapy (two received doxorubicin, one received mitoxantrone).[34] A dog treated with pericardiectomy, intrathoracic and intravenous cisplatin, and intravenous doxorubicin remained free of disease at 27 months.[35] In a report of eight dogs with pericardial mesothelioma, the median survival time was 60 days (ranging from 15 to 300 days) postpartial pericardiectomy. The one dog that survived 300 days was treated with doxorubicin and intracavitary cisplatin for the 4 months preceding death.[27] Thoracoscopic partial pericardiectomy is a less invasive procedure than open thoracic surgical pericardiectomy and has been successfully performed in dogs with malignant pericardial effusions, including four dogs with mesotheliomas.[36] Median survival for animals with mesotheliomas in any location that are not treated is difficult to assess from reports as the tumors are rare and animals frequently are euthanized at the time of diagnosis.

Intracavitary cisplatin has shown palliative potential in the dog; it was well tolerated and greatly decreased mesothelioma-associated thoracic fluid accumulation in three dogs in one study.[37] The treatments also appeared to arrest tumor growth for a limited time. Unfortunately, penetration of the chemotherapy is only to a small depth (2 to 3 mm), and thus large masses will not be affected significantly. In such cases, combining debulking surgery or systemic chemotherapy, such as doxorubicin or mitoxantrone, with intracavitary cisplatin may be of benefit.

COMPARATIVE ASPECTS

In people, mesothelioma is closely linked to exposure to aerosolized asbestos fibers. Approximately 80% of cases have a history of occupational exposure, with the type of employment significantly affecting relative risk.[38,39] Occupations such as construction work, ship building, heating trades, asbestos mining, and insulation work are strong risk factors in the development of mesothelioma in people.[38] Family members of exposed industrial workers are at risk due to asbestos fiber exposure from the workers' clothing. Affected individuals routinely have greatly increased counts of asbestos fibers in parenchymal lung tissue.[39] The latency period from time of exposure to tumor development is long, with reports ranging from 12 to 50 years.[38] Other risk factors discussed in the development of mesotheliomas include past radiation, exposure to certain chemicals or nonasbestos fibers, and genetic tendency.[12,38] In addition, the DNA tumor virus Simian 40 (SV40) is suspected to act as a cocarcinogen with asbestos in causing mesotheliomas.[13,40,41] SV40 was introduced into a large percentage of people through contaminated polio vaccines between 1955 and 1963. SV40 can transform cells and experimentally leads to mesothelioma development in lab animals. However, one study presents strong evidence against the proposed connection between SV40 and mesothelioma and shows laboratory support that the high prevalence of SV40 found in human mesotheliomas in previous studies are likely due to false positive-prone molecular assays.[42]

In humans, the median survival is approximately 1 year from symptom onset. There is no consensus in the literature regarding therapy, as data are lacking to support any single or combination approach as significantly improving survival time or quality of life over supportive care.[14] In select patients with very confined disease of the epithelial type, aggressive multimodality therapy, including surgery, radiation, and systemic chemotherapy, can extend survival (median 51 months).[12] In nonsurgical patients, a multitude of chemotherapy agents and protocols have been tried. Vinorelbine single-agent therapy has shown a 24% partial response rate and a 40% improvement in quality of life.[14] Pemetrexed, a multitargeted antifolate, combined with cisplatin showed significantly increased survival times and quality of life parameters compared to cisplatin alone; this combination is the new standard of care for advanced mesothelioma and is being investigated in the adjuvant setting.[43] Numerous cytokines (e.g., IL-2, α-2b interferon) have been studied and shown benefits in therapy of bulky disease. New approaches include investigation of growth factor receptor inhibitors (e.g., Avastin, Iressa) in ongoing clinical trials.[14] Microarray studies of mesotheliomas using the gene expression ratio technique have shown great benefit in both confirming a diagnosis and in prognostication for survival time; such tools will help to tailor therapies in the future.[44]

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SECTION E
Neoplasia of the Heart

William C. Kisseberth

INCIDENCE AND RISK FACTORS

Neoplasms affecting the pericardium, heart base, or the myocardium are infrequent in the dog and even more rare in the cat. In necropsy series, primary or metastatic cardiac tumors represented 0.12% to 5.73% of canine cases submitted for necropsy,[1-9] and 11.74% to 28.3% of all neoplasms involved intrapericardial tissues.[1,2] The only primary tumors of the cardiovascular system found in a series of 4933 feline necropsies were one case of mesothelioma of the pericardium and two cases of chemodectoma.[10] A Veterinary Medical Database (VMDB) search identified 1383 dogs with tumors of the heart from a population of 729,265 (overall incidence 0.19%).[11] A similar search of the VMDB revealed 58 cats with cardiac tumors from a total of 210,388 cats seen (overall incidence 0.0275%).[12] Cardiac tumors (excluding lymphoma) occur most frequently in middle-aged to older (7 to 15 years) dogs.[11] Breeds reported to be at increased risk or predisposed for cardiac hemangiosarcoma include the German shepherd dog and golden retriever.[2,13,14] Aortic body tumors occur most commonly in older brachycephalic dogs,[2,15-17] including boxers, Boston terriers, English bulldogs, and German shepherd dogs.[11] It has been suggested that chronic hypoxia may stimulate development of chemoreceptor tumors in both dogs and humans[15,16] and that this factor could explain the increased occurrence of aortic body tumors in brachycephalic breeds. Based on the VMDB study, 12 breeds were identified as having a significantly higher incidence of cardiac tumors rate compared to all other breeds. Surprisingly, 11 breeds had a higher incidence rates than the German shepherd dog, including the golden retriever, which had a higher incidence rate and total number of recorded tumors.[11] Presumably, the fact that some of these breeds are less popular may account for their infrequent appearance in literature reports. The most common cardiac tumor in cats is lymphoma.[12]

PATHOLOGY AND NATURAL BEHAVIOR

Neoplasms affecting the heart may occur in intracavitary, intramural, or pericardial locations, or at the heart base. Primary tumors may be benign or malignant, with most primary tumors in the dog occurring in the right atrium and auricle (Figure 32-21). The most common primary heart tumor in the dog is hemangiosarcoma, followed by aortic body tumor (chemodectoma, paraganglioma.[1-12] Cardiac hemangiosarcomas often are
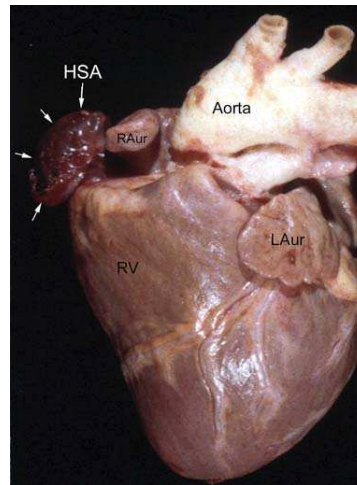
Figure 32-21

Postmortem specimen of a canine heart demonstrating hemangiosarcoma (*HSA*) of the right auricle (*RAur*). The tumor arises from the auricular wall and extends into the pericardial space (the pericardium has been removed). Vascular compartments of these tumors frequently rupture, leading to hemopericardium and cardiac tamponade. *LAur*, left auricle; *RV*, right ventricle. (Image courtesy of John Bonagura, DVM, DACVIM and Matthew W. Miller, DVM, DACVIM).

associated with hemorrhagic pericardial effusion, cardiac tamponade, and metastatic disease.[13,14,18,19] Aortic body tumors arise from chemoreceptor cells at the heart base. These tumors are primarily locally invasive but occasionally metastasize. Other primary cardiac tumors reported in the dog include lymphoma, undifferentiated sarcoma, myxoma, ectopic thyroid carcinoma, fibroma, fibrosarcoma, rhabdomyosarcoma, chondrosarcoma, mesothelioma, granular cell tumor, osteosarcoma, and myxosarcoma.[20-39] In contrast to people, primary heart tumors are more common than metastatic tumors in the dog.[11] This is due largely to the high incidence of hemangiosarcoma in the dog. Hemangiosarcoma accounts for 40% to 69% of cardiac neoplasms in the dog;[1,2,11] however, in most studies, cardiac hemangiosarcoma has been considered a primary site, although most dogs have evidence of disease at other sites at the time of diagnosis and it usually is impossible to determine which is the primary site and which are the metastatic sites. Consequently, if most of the hemangisarcoma diagnoses were considered multicentric or metastatic, then metastatic tumors would be more frequent. Metastatic mammary carcinoma, melanoma, mast cell tumor, malignant histiocytosis, pheochromocytoma, and granulosa cell tumor have been reported in the dog.[40-44] Cardiac hemangiosarcoma, aortic body tumors and myxoma have been reported in the cat;[12,45,46] however, metastatic tumors are more

common, with lymphoma, mammary gland carcinoma, pulmonary carcinoma, salivary gland adenocarcinoma, oral melanoma, rhabdomyosarcoma, sweat gland adenocarcinoma, oral squamous cell carcinoma, and mast cell tumor having been reported.[10,12,47-51]

HISTORY AND CLINICAL SIGNS

Tumors involving the heart cause varied clinical signs. Cardiac tumors disrupt the normal function of the tissues from which they arise, leading to altered cardiovascular function. In general, signs result from (1) the physical presence of the mass causing obstruction of blood flow into or out of the heart, (2) external compression of the heart that impedes filling (e.g., pericardial effusion and resulting cardiac tamponade), and (3) disruption of normal heart rhythm or contractility if myocardial infiltration occurs or ischemia develops.[12] As such, clinical signs produced by cardiac tumors are more closely related to their precise anatomic location than their histologic type. Specific clinical signs observed in an individual patient are influenced by the tumor's size and location and the presence of pericardial effusion. Acute death from rupture of tumor with subsequent blood loss, with or without cardiac tamponade, is a common sequela of cardiac hemangiosarcoma. Ironically, bleeding from tumor within the heart chambers is not a clinical problem since hemorrhage occurs within the vascular space. Sudden death due to cardiac arrhythmia may also occur. Cardiac hemangiosarcoma, as well as the majority of reported primary sarcomas of the right heart in dogs, generally produce signs of right heart failure due to the presence of cardiac tamponade and a preponderance of right-sided masses causing inflow obstruction. These signs include ascites, pleural effusion, jugular venous distension, abnormal jugular pulsations, exercise intolerance, dyspnea, pulse deficits, muffled heart sounds, and syncope. Tumors that cause pericardial effusion and cardiac tamponade have been reported most often.[11] Cardiac or pericardial tumors are responsible for approximately 60% of cases of pericardial effusion in dogs.[52] The most common tumors to cause pericardial effusion are right atrial hemangiosarcoma, aortic body tumors, and mesothelioma.[52]

Associated clinical signs resulting from cardiac tamponade include restricted ventricular filling secondary to external cardiac compression, venous congestion, and poor cardiac output. Heart base tumors are most often associated with pericardial involvement of the tumor and accompanying pericardial effusion.[12] Edema, ascites, cough, dyspnea, weight loss, and vomiting are the signs most commonly reported with aortic body tumors in dogs.[12,17,53] Heart base masses are a common cause for cranial vena cava syndrome (edema of the head, neck, and forelimbs) due to tumor pressure on the cranial vena cava. Sometimes these tumors may be present without causing clinical signs, or are incidental findings at necropsy. Cardiac tumors that do not cause pericardial effusion can cause signs of congestive heart failure or low cardiac output by obstructing blood flow within the heart or great vessels and by inducing arrhythmias. Syncope and weakness with exertion or excitement are common signs in animals with cardiac tumors. These signs of low cardiac output can result from cardiac tamponade, blood flow obstruction, arrhythmias, impaired myocardial function, and hemorrhage.[11] Clinical signs may be absent if the tumor is small or in a location that does not affect cardiac function.

DIAGNOSTIC TECHNIQUES AND WORKUP

The diagnosis of cardiac neoplasia in the dog and cat is usually based on clinical history, physical examination, and radiographic and echocardiographic findings. In most instances, cytologic or histologic confirmation of neoplasia is not obtained antemortem; however, a fine-needle aspirate or biopsy may be obtained when indicated and technically feasible. An electrocardiogram may be normal in patients with cardiac tumors or may show any of a variety of arrhythmias (Fig. 32-22), which may correlate with the underlying site of the primary or metastatic tumor or may be secondary to myocardial ischemia or hypoxia. Low-amplitude QRS complexes and electrical alternans may be seen in animals with pericardial effusion.[54] Sinus tachycardia is common with cardiac tamponade. Animals with a large volume of pericardial effusion may have a rounded ("globoid")

Figure 32-22

Lead II ECG from a dog with a large pericardial effusion. Overall QRS voltages are relatively small (<1 mV) with periods of electrical alternans evident. The ECG findings in pericardial effusion are inconsistent. Electrical alternans is a relatively specific, but insensitive indicator of pericardial effusion in dogs. This ECG finding is usually observed when the effusion is large and allows the heart to swing within the pericardial space. ECG recorded at 50 mm/s; 10 mm = 1 mV. (Image courtesy John Bonagura, DVM, DACVIM.)

cardiac silhouette. Smaller fluid accumulations may allow visualization of atrial and tumor shadows.[12] Thoracic radiographs may reveal cardiomegaly or effusions associated with cardiac tamponade. Mass lesions, if seen, are most common in the areas of the right atrium and heart base. Lung metastases may be seen.

Echocardiography is the most valuable diagnostic procedure for identifying tumors of the heart in cats and dogs[55-57] (Figure 32-23). In one study of histologically confirmed hemangiosarcoma of the right atrium/auricle, two-dimensional echocardiography had a positive predictive value of 92% (11/12) and a negative predictive value of 64% (9/14) in dogs.[57] That is, false-negative studies were common. Tumor location (extrapericardial, noncavitary pericardial, and small auricular masses) and size appear to be the most important factors for false-negative results with echocardiography.[57] Pericardial effusions are a common finding associated with cardiac tumors in both cats and dogs.[35,57,58]

Other clinical diagnostic methods for the evaluation of cardiac or pericardial masses include pneumopericardiography (for historical perspective), selective and nonselective angiography, gated radionuclide imaging, and endomyocardial biopsy in selected patients.[52,59,60] Cytologic evaluation of pericardial fluid and pericardial fluid pH has proven to be of limited usefulness in diagnosing or discriminating between neoplastic and non-neoplastic causes of pericardial effusion.[61,62]

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are sensitive and specific markers for myocardial ischemia and necrosis. Dogs with pericardial effusion had significantly higher concentrations of cTnI, but not cTnT, than normal dogs. Furthermore, dogs with cardiac hemangiosarcoma had significantly higher concentrations of cTnI than did dogs with idiopathic pericardial effusion.[63]

Every effort should be made to determine the extent of disease and the existence of primary or metastatic sites elsewhere in the patient. A minimum database of a complete blood count, serum biochemical profile, coagulation profile, thoracic radiographs, and abdominal ultrasound or radiographs should be obtained in patients diagnosed with cardiac masses. If no other evidence of neoplastic disease is found, then surgical exploration with biopsy and attempted resection may be indicated.

THERAPY

Treatment of patients with cardiac tumors consists of treating existing arrhythmias and clinical signs of heart failure, if present. Unfortunately, without effective antitumor treatment the hemodynamic consequences of the mass often are refractory to medical management. Surgical resection may be indicated in a small number
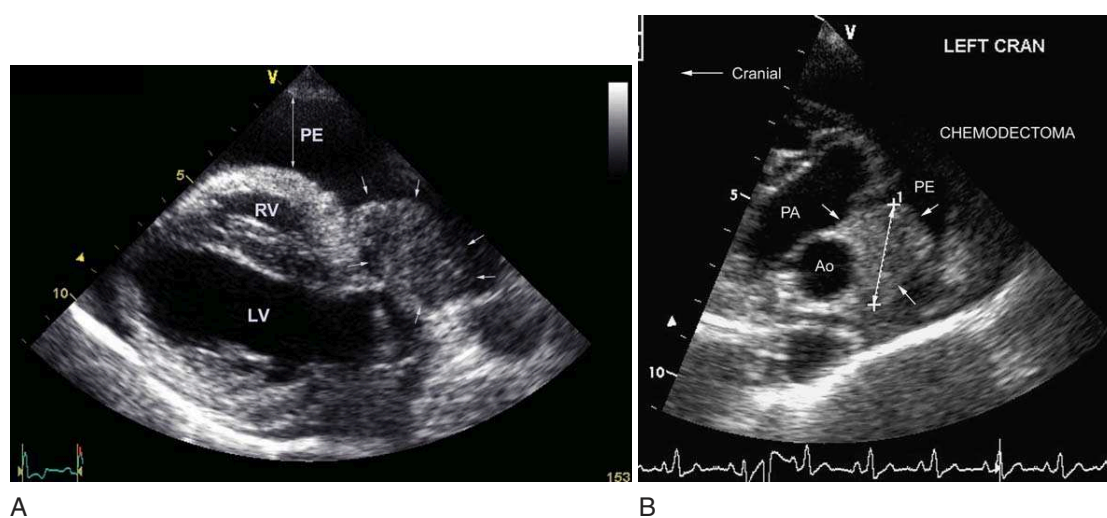
Figure 32-23

A, Two-dimensional echocardiogram obtained from the right intercostal position in a dog with cardiac hemangiosarcoma. A pericardial effusion (*PE*) is evident in the near field. The mixed-echoic mass (*small arrows*) is attached to the right atrial wall and straddles the atrioventricular junction. These are typical locations for cardiac hemangiosarcoma. The right atrial cavity is collapsed owing to cardiac tamponade. *RV*, right ventricle; *LV*, left ventricle. (Image courtesy John Bonagura, DVM, DACVIM). **B,** Two-dimensional echocardiogram obtained from the left cranial thorax of a dog with a chemodectoma (aortic body tumor). The mass (*arrows*) is adjacent to the aorta (*Ao*) and is contrasted in the near field by a small pericardial effusion (*PE*). Detection of cardiac tumors by echocardiography often requires off-angled image planes as demonstrated here. A segment of the pulmonary artery (*PA*) is observed. The maximum length of the tumor also is indicated between the cross-hairs. (Image courtesy John Bonagura, DVM, DACVIM).

of primary cardiac tumors.[13,19,25,64,65] Surgical resection of right auricular masses in dogs with cardiac hemangiosarcoma must be considered a palliative procedure due to the high probability of metastatic disease. Narrow based or pedunculated lesions would be most amenable to safe resection, but distinguishing them from broad-based and invasive lesions preoperatively is difficult. Surgery should be considered if a "solitary" lesion is present and not part of diffuse metastatic disease and if the patient is refractory to pericardiocentesis. Surgery, if performed, should also include a subphrenic pericardectomy as palliation. Dogs with aortic body tumors generally benefit from pericardectomy, independent of the presence or absence of pericardial effusion at the time of surgery.[17,53] Both open and closed thoracic techniques have been described for palliative pericardectomy and pericardiotomy.[66-70] Thoracoscopy has been recommended as an alternative to open thoracotomy for biopsy and pericardectomy, since most lesions are unresectable. Thoracoscopy requires advanced training and special instruments and equipment if it is to attain the goals of tissue diagnosis, decreased operative time, and decreased morbidity.[66-68] These procedures are palliative and result in short-lived benefit in most instances. Doxorubicin-based chemotherapy protocols, including doxorubicin alone, doxorubicin and cyclophosphamide, and doxorubicin-cyclophosphamide-vincristine have been used alone[71] and in combination with surgery[19] for the treatment of cardiac hemangiosarcoma.

PROGNOSIS

The prognosis for primary cardiac tumors generally is poor. Most cases respond poorly to medical management. Reported mean survival times of dogs with cardiac hemangiosarcoma treated with surgical resection alone range from 46 days to 5 months.[13,19,64] Survival time was significantly longer in dogs treated with tumor resection and adjuvant chemotherapy, with a reported mean of 164 days,[19] comparable to dogs with splenic hemangiosarcoma treated with splenectomy and adjuvant chemotherapy.[72] Surgical resection of aortic body tumors usually is not possible; however, dogs that received a pericardectomy survive longer (median survival 730 days) than those that did not have a pericardectomy (median survival 42 days).[53] Tumors found at exploratory thoracotomy commonly are judged to be unresectable; however, a few cases with longer disease-free intervals following resection of tumors other than hemangiosarcoma have been reported.[25,27] Some histologies are associated with a more favorable prognosis. In one study, dogs with hemangiosarcoma involving the pericardium survived a median of 16 days, while those with mesothelioma survived a median of 15.3 months following surgery.[73]

COMPARATIVE ASPECTS

In people, primary tumors of the heart and pericardium are rare. The vast majority of tumors involving the heart and pericardium are metastatic.[74-76] They occur with a frequency of 0.001% to 0.28% in reported or collected postmortem series.[77] Primary tumors are usually cavitary and 75% are benign. Familial cardiac myxomas occur and appear to have an autosomal dominant transmission and are caused by mutations in the PRKAR1∝alpha gene that encodes a regulatory subunit of protein kinase A.[75,78] Myxomas constitute nearly 50% of all histologically benign tumors of the heart.[75,77] Seventy-five percent of myxomas occur in the left atrium. Systemic tumor embolization occurs with high frequency.[75] Surgical resection of myxoma is the treatment of choice, with recurrence of sporadic atrial myxomas being rare following excision.[75] Rhabdomyoma is the most common cardiac tumor of infants and children.[77] Other benign primary cardiac tumors that have been reported in people include fibroma, papillary fibroelastoma, lipoma, cystic tumors of the atrioventricular node, hemangioma, lymphangioma, and intrapericardial paraganglioma.[75]

Almost all primary malignant cardiac tumors are sarcomas, most frequently angiosarcomas.[75,76,79] As is the case with hemangiosarcoma in the dog, angiosarcoma of the heart in people most commonly originates in the right atrium or pericardium.[75] Rhabdomyosarcoma and mesothelioma rank second and third, respectively, in frequency among primary malignant tumors of the heart and pericardium in people. Palliative and local control of malignant primary tumors can be achieved with extensive resection.[80,81] Adjuvant chemotherapy and radiation therapy have been used; however, their routine use for primary cardiac sarcomas has been questioned.[82] Cardiac transplantation has been utilized on occasion.

Metastatic tumors involve the heart and pericardium 20 to 40 times more frequently than primary tumors.[77] Incidence rates of 3.4% and 5.7% for myocardial metastases were reported in two large autopsy studies of patients who died of cancer.[75] Malignant melanoma metastasizes most frequently to the myocardium, accounting for more than 50% of cases.[75] Cardiac metastasis also frequently occurs with bronchogenic carcinoma and carcinoma of the breast.[77]

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SECTION F
Histiocytic Diseases

Craig A. Clifford and Katherine A. Skorupski

BACKGROUND

The histiocytic proliferative disorders (HPDs) in the canine encompass a wide spectrum of diseases characterized by a variety of biologic behaviors. Previously, a paucity of information existed in the veterinary literature regarding these disorders, limited to case reports and a few retrospective studies. Due to the continued development and application of more reliable immunophenotyping reagents aiding in identifying a cellular origin, a clearer picture is evolving.[1] This research has introduced new terminology to describe the vast array of disease syndromes based on distribution and cell lineage. In spite of this research, the etiology and pathogenesis of these diseases is largely unknown, and they remain a controversial area in veterinary medicine. They are functionally divided into dendritic cell tumors and macrophage tumors.[2-4] Subdivisions are based on lesion distribution (localized versus disseminated).[3] Overall, they represent less than 1% of all tumors affecting lymphoid tissue.

The HPDs are a frustrating group of diseases because the clinical presentation, behavior, and responsiveness to therapy vary tremendously. Furthermore, with standard paraffin sections, it is often difficult to differentiate them from granulomatous, reactive inflammatory diseases, or lympho-proliferative disease.[4] Lastly, more than one form may be present within a specific tissue section, adding to the confusion.[5] At the present time, three well-defined HPDs have been recognized in the canine. These include cutaneous histiocytoma, reactive histiocytosis (cutaneous and systemic histiocytosis), and histiocytic sarcoma (localized and disseminated also known as malignant histiocytosis).[3,6-15] A sufficient grasp of these syndromes predicates a basic understanding of

the lineage of these cells. In general, two major subsets of cells were thought to make up this neoplasm: the antigen-presenting cells (dendritic cells) and the antigen-processing cells (phagocytic cells).[3] Although these cells originate from the bone marrow and share a common precursor, the antigen presenter and processors are believed to represent two parallel and independent lines of differentiation.[3,4]

The term *histiocyte* encompasses a vast array of cells including the differentiated cells of the monocyte/macrophage lineage (sinusoidal macrophages of the spleen, alveolar macrophages, and Kupffer cells); Langerhans cells of the skin; interdigitating dendritic cells (DC) of lymph nodes, thymus, and spleen; and dendritic reticulum cells found in germinal centers of lymph nodes (Figure 32-24).[16,17] All except the macrophage arise from bone marrow CD34+ committed stem cell precursors that differentiate into several DC lineages. This differentiation process is likely controlled by a variety of colony stimulating factors (CSF) such as granulocyte-macrophage (GM)-CSF and macrophage (M)-CSF for macrophages and tumor necrosis factor (TNF)-alpha, GM-CSF, interleukin (IL)-3, IL-4 for DC's.[18-23] Macrophages are the differentiated cell product of peripheral blood monocytes. They are called "professional phagocytes" with a high degree of lysosomal enzyme content. Their main function is to act as immune defenders against microorganisms and to remove organic and inorganic particles. The DCs are a heterogenous population of cells, which includes intraepithelial DC (Langerhans cells (LC)) present in the skin and mucosal epithelia, follicular DC present in germinal centers of lymphoid tissue, interstitial DC present in nonlymphoid organs and interdigitating DC present in the T cell rich areas in lymphoid tissue.[3,4] Dendritic cells are considered poor phagocytes with low levels of lysosomal enzymes. They are called "professional antigen presenting" cells, as they are highly evolved in regards to antigen presenting ability. Specifically, they are responsible for the initiation of the immune response. Their main function is to educate naïve T cells during the induction of the primary immune response and to serve as sentinels for recognition of antigen.[4] Processed antigens are presented in context with major histocompatibility complex (MHC)-1 molecules, MHC class II molecules and CD1 molecules to naïve T cells leading to their activation. Other co-stimulatory factors needed for the induction of an immune response include B7 family-CD80 and CD86 present on dendritic APC, and the CD28 and CTLA-4 associated ligands present on T cells.[4,18 24,25]

Immunophenotypic and morphologic characterization of DCs can aid in their subset differentiation. For determination of histiocytic origin CD1 (marker of APCs), CD11b (mediates phagocytosis of particles opsonized with complement, leukocyte adhesion molecule), CD11c
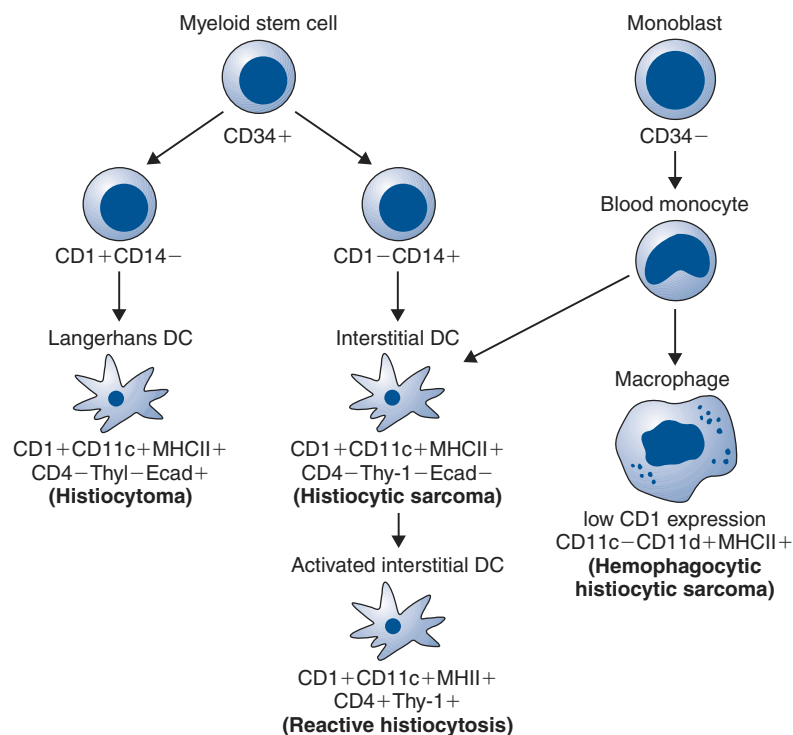
Figure 32-24

Lineage of histiocytic cells and the corresponding histiocytic disease syndromes.

(clears opsonized particles and immune complexes, binds fibrinogen, leukocyte adhesion molecule), CD18 (important in adhesion and signaling), CD14 (receptor for endotoxin), CD68 (monocyte marker), and MHC class II can be utilized. [1,3,4,11,26,27] To further differentiate between DC and macrophage origin, CD1 and CD11c expression (DC) and CD11b, CD 11d and CD68 expression (macrophage) is evaluated. [3,411,28-30] Further distinction between DC as either LC or interstitial DC is possible via expression of E-cadherin present on LC and CD 90 present on interstitial DC. [4] Unfortunately the use of such markers generally requires frozen tissue sections, which is limited to specific facilities. Standard paraffin sections, however, in conjunction with a panel of markers can aid in cellular identification (see Figure 32-24). [4] Specifically, E-cadherin, CD79a (B cell), CD3 (T cell), CD45 (leukocytes), CD45RA (leukocytes), lysozyme (high labeling associated with macrophage origin), CD11d, and CD18 may be useful in this setting. [1,4] Langerhans cells contain distinctive Birbeck granules that are not noted in macrophages. These granules are characterized as rod shaped organelles with "zipper-like" striations. [16,31]

As previously stated, the three major HPDs recognized in the dog are cutaneous histiocytoma, reactive histiocytosis (cutaneous and systemic), and histiocytic sarcoma (localized and disseminated). Although not truly considered part of the HPDs, splenic histiocytosis/fibrohistiocytic nodules/malignant fibrous histiocytoma will be briefly discussed in a limited manner. Cutaneous histiocytoma, a benign entity primarily affecting young dogs, is discussed in depth in Chapter 18 and is only briefly discussed here. The remainder of the discussion focuses on reactive histiocytosis and histiocytic sarcoma.

CUTANEOUS HISTIOCYTOMA

Cutaneous histiocytoma (CH) is a benign tumor that often occurs as a single lesion in young dogs (<3 years of age); however, dogs of any age can be affected. These tumors typically present as a single solitary lesion, often in the cranial portion of the body. The growth of the lesion can be quite rapid (1 to 4 weeks), and oftentimes these lesions spontaneously regress within 1 to 2 months of presentation. [7,8,32-38] The regression is lymphocyte mediated. Multiple tumors and metastatic histiocytomas have been reported, however, this latter form appears to be related to a syndrome in humans termed Langerhans cell histiocytosis (LCH). [38-41] This LCH syndrome was reported in an 8-month-old Daschund puppy. [42] Studies have demonstrated histiocytomas are of epidermal Langerhans cell origin via expression of CD1a, CD1b, CD1c, CD11c, E-cadherin, MHC class II molecules and inconsistent lysozyme immunoreactivity and negative expression of Thy-1 and CD4 (marker of activated DC). [1,3,4,7,8,11] The expression of E-cadherin is unique to

histiocytomas and negative expression of Thy-1 and CD4 help to differentiate histiocytomas from reactive histiocytosis (CH, SH). [3,4,11] It has been suggested that the term *epidermotropic Langerhans cell histiocytosis* may better reflect this disease process. [4] A more detailed discussion on cutaneous histiocytomas is presented in Chapter 18.

REACTIVE HISTIOCYTOSIS

Reactive histiocytosis can be separated into cutaneous histiocytosis (CHS) and systemic histiocytosis (SH). Cutaneous histiocytosis represents a benign, diffuse aggregation of histiocytes that grows rapidly into infiltrating nodules, plaques, and crusts within the skin and subcutaneous tissue. [4,9,11,38] This disease tends to occur in younger dogs; however, one study noted a range of 2 to 11 years. A breed predisposition has yet to be identified. A study of 18 dogs with CHS noted a male predilection. [11] This disease is limited to the skin and subcutis, but it can be multifocal. The head, pinna, limb, and scrotum are commonly reported sites. [4,9,11,38] Lesions may also be found on the nasal planum and within nasal mucosa, the gross appearance of which has been described as a "clown nose" (Figure 32-25). [41] Histologically, lesions contain a pleocellular histiocytic infiltrate, often perivascular within the dermis and subcutaneous tissue. Lymphoid infiltrates (T cell predominance) and some neutrophils are common. Vascular invasion may be present. These histiocytic cells express CD1a, CD1b, CD1c, CD11c, MHC class II molecules, Thy-1, and CD4 but are negative for E-cadherin. The expression of Thy-1 and CD4 aids in differentiating this disease, which appears to be of interstitial cell origin from CH, which is of epidermal LC origin. [4,9,11,38] CHS follows a benign course and is often responsive to immunosuppressive therapy, although spontaneous regressions have been reported. In a study seven dogs treated with systemic steroids, partial responses were seen in the majority of dogs. [11] Remission was reported in one dog receiving intralesional corticosteroids. Spontaneous regression occurred in 2 of 13 dogs, and surgery was curative in another. [11] Response to steroids appears variable, but most dogs will have a partial response (Figure 32-26). [4,9,11] Long-term maintenance therapies may be warranted to prevent recurrence; however, affected dogs may have a prolonged survival. In refractory cases, other immunosuppressive agents may be useful including cyclophosphamide, Cyclosporine A, and azathioprine. Of importance is the need for patient evaluation to ensure the disease is CHS and not SH.

Systemic histiocytosis is a non-neoplastic disease of proliferative lymphocytes occurring in Bernese mountain dogs, rottweilers, golden retrievers, and Irish wolfhounds. [4,10-12,38,43,44] In the Bernese mountain dog, this appears to be a familial disease, suggesting a genetic predisposition. [4,10-12,38] Earlier work also noted a male

A   B
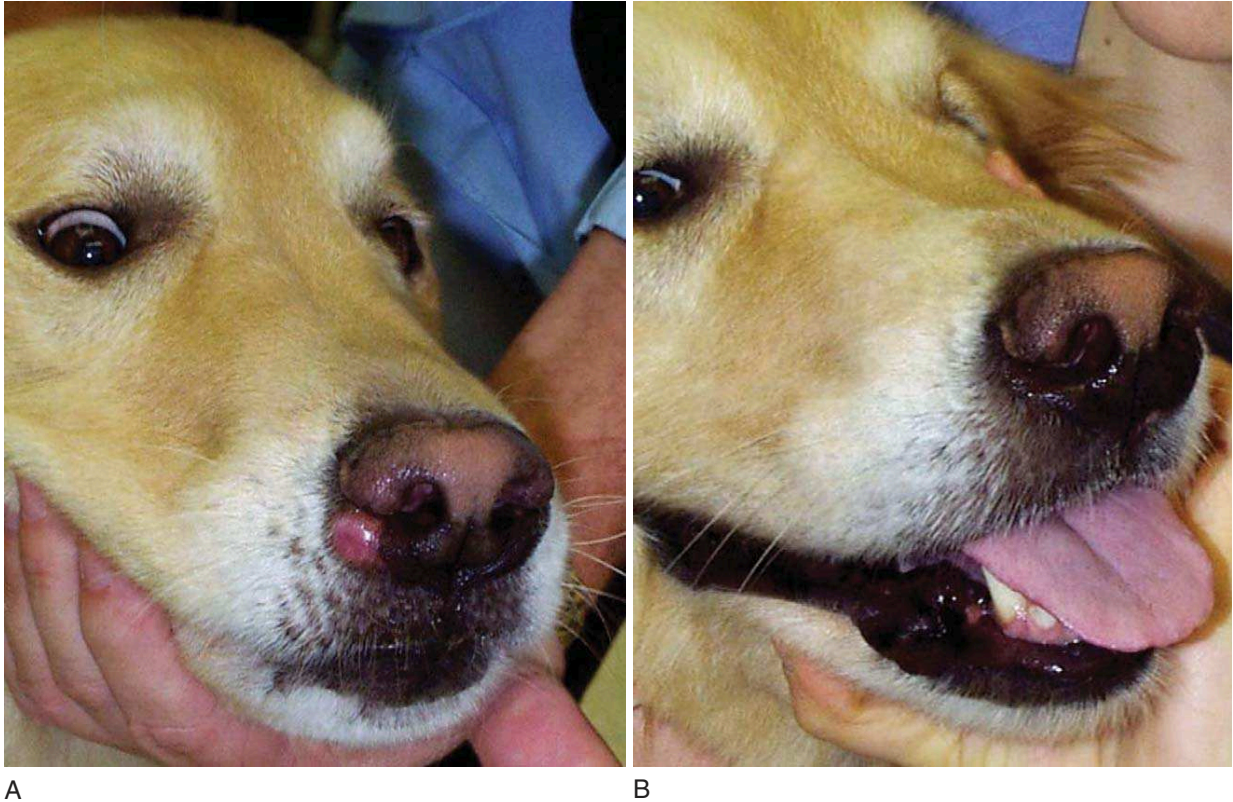
Figure 32-25

A nasal planum lesion **(A)** in a 14-month-old golden retriever with cutaneous histiocytosis. This and other lesions (Figure 32-26) completely regressed **(B)** following prednisone and one cyclophosphamide treatment. The cyclophosphamide was given 1 week after initiation of prednisone as the regression rate was initially very slow. He was eventually weaned off of all drugs and remains in remission.

A   B
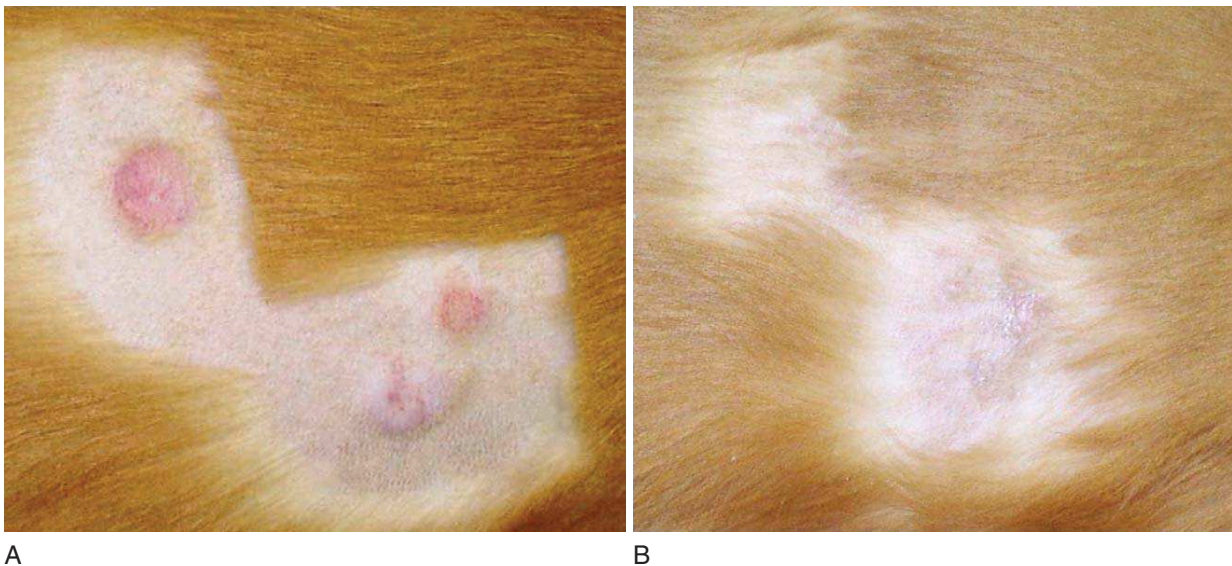
Figure 32-26

A, Cutaneous histiocytosis in a 14-month-old golden retriever. This and other lesions completely regressed **(B)**, following prednisone and one cyclophosphamide treatment. The cyclophosphamide was given 1 week after initiation of prednisone as the regression rate was initially very slow. He was eventually weaned off of all drugs and remains in remission.

predilection in the Bernese breed.[10-12]The age of onset for SH is 3 to 9 years. Dermal lesions manifest in the skin with similar site predilection as CHS; however, other sites, including subcutaneous tissue, lymph node, bone marrow, scrotum, spleen, liver, lung, and mucous membranes (nasal and ocular tissue) occur. Ocular involvement may affect the conjunctiva, sclera, ciliary body, extraocular muscles, and retrobulbar tissue.[44] One distinguishing feature from CH is the presence of palpably enlarged peripheral lymph nodes and organ involvement.[4,11,38] Clinical signs vary depending on the affected tissue and severity of disease; however, depression, anorexia, weight loss, conjunctivitis, and harsh respiration are common. In one study, 2 of 26 dogs were hypercalcemic at presentation.[11] This disease appears similar to some forms of the Langerhans cell histiocytosis in humans, which are reactive disorders likely secondary to immune system dysregulation. Clinicopathologic features of SH are varied; however, anemia, monocytosis and lymphopenia are consistently reported.[31,38]

Cytologically, SH lesions are similar to granulomatous inflammation or CHS, characterized by a predominance of benign histiocytes with occasional multinucleated giant cells.[31] Other inflammatory cells including lymphocytes, eosinophils, and neutrophils can be interspersed. Erythrophagia is reported but rare. Histiocytic cells are large, contain voluminous cytoplasm, and have indented nuclei with variable nucleoli.[31] Histologically, these lesions are characterized by multicentric, nodular, angiocentric histiocytic infiltrates within the deep dermis and panniculus. To a lesser degree lymphocyte, plasma cell, eosinophil and neutrophil infiltration is present, with small lymphocytes making up the greatest proportion of nonhistiocytic cells.[4,10-12,38] Blood and lymphatic vessel invasion may also be noted. In some cases, vascular wall degeneration, thrombosis, and ischemic necrosis may be present.[4,11,38] The histologic appearance of lesions within other organs consists of nodular, perivascular accumulation of histiocytes, lymphocytes, and neutrophils.[3,4,10-12] With immunohistochemistry, SH lesions express CD1, CD11c, MHC class II, Thy-1 and CD4, similar to that of CHS.[3,4] This expression pattern suggests these cells are of activated interstitial DC and not epidermal LC (histiocytoma).[1,3,4] The majority of the small lymphocytes present within the lesions have been demonstrated to be of T cell origin (CD3 and TCRαβ positive) and 50% were CD8 positive.[3,4] Unlike cutaneous histiocytomas, the presence of T cells is not associated with regression but likely secondary to cytokine induced migration. Interestingly, ocular involvement appears similar to lesions described with fibrous histiocytoma.[31,44]

Lesions may have a waxing and waning presentation but generally do not spontaneous resolve and thus require long-term therapy. Corticosteroids alone appear ineffective in controlling this disease long term.[4,10-12,38,43,44]

Experimentally, bovine thymosin fraction-5 demonstrated some efficacy in two dogs.[4,12] The use of azothioprine, cyclosporine A or leflunomide (Hoechst Marion Roussel, Wiesbaden, Germany) has yielded long-term control in some cases.[4,11] Cyclosporine and leflunomide both have the ability to inhibit T cells. The successful treatment with either agent suggests a significant role of T cell lymphocytes in this disease. Two proposed mechanisms for immune dysregulation include increased local cytokine (GM-CSF, IL-4, TNF) production by T cells due to persistent dendritic cell accumulation and inappropriate dendritic and T cell interaction due to abnormal regulation of accessory ligands on both cell types.[4,11] These molecules are needed for induction of the immune response and the subsequent down regulation of the response. Although no infectious cause has been identified, it is important to rule out such etiologies with either a culture or immunohistochemistry of histopathology samples.[4,11] The clinical course of this disease is often prolonged but rarely results in death. Generally, there are episodic periods of response followed by recrudescence with most dogs euthanized due to repeated relapses in clinical condition or failure to respond to therapeutics.[4,11,12,38]

HISTIOCYTIC SARCOMA

Pathology and Natural Behavior

Malignant proliferations of histiocytic cells were first reported in the dog in the late 1970s. A predisposition in Bernese mountain dogs was reported in 1986 in a group of 11 dogs, 9 of which were related.[45] In that study group, a male predisposition was also noted (10 of 11 dogs), and the majority had pulmonary involvement. Histiocytic sarcoma (HS) has since been identified in a variety of breeds; however, flat-coated retrievers and rottweilers appear to be overrepresented. Dogs are commonly middle aged or older, but HS has been reported in dogs as young as 3 years of age. HS may present with either localized organ involvement or disseminated, multiorgan involvement. *HS* is currently the preferred term for identifying malignant tumors of antigen presenting dendritic cell origin, and the older term *malignant histiocytosis* refers to the disseminated form of the disease. Reported anatomical sites include lung, lymph node, liver, spleen, stomach, pancreas, mediastinum, skin, skeletal muscle, central nervous system, bone and bone marrow, and the authors have seen cases with intranasal and ocular involvement.[45-49] In a clinical population, 5% of primary brain tumors were HS and, in a necropsy population, 4.5% of secondary brain tumors were HS.[50,51] In addition, 18 of 35 tumors of synovial origin previously diagnosed as synovial cell sarcomas were reclassified as HS based on

immunohistochemial expression of CD18.[49] Eleven of these 18 dogs were rottweilers.

It has been proposed that a subtype of histiocytic sarcoma exists. Termed "hemophagocytic HS," these tumors are differentiated by their immunohistochemical staining pattern consistent with macrophages (CD11d+, CD11c–, MHCII+, minimal CD1 expression), not dendritic cells.[4] Clinically, these tumors have been seen in the spleen and bone marrow and may behave more aggressively due to their cellular ability to phagocytose material including host red blood cells.

History and Clinical Signs

Presenting complaints and clinical signs vary depending on site(s) of involvement by the tumor, but nonspecific symptoms such as lethargy, inappetence, and weight loss are common. Other signs include cough and dyspnea, fever, vomiting, diarrhea, and lymphadenopathy.[45,47] Lymphadenopathy may be the only clinical sign and can appear at a site distant to other tumor lesions. Lymphadenopathy may not be associated with local lymph node drainage from a primary tumor. Patients may also present with clinical signs related to severe anemia or thrombocytopenia, especially in tumors with cellular phagocytosis.

Diagnosis and Staging

Diagnosis can be obtained via cytologic or histologic examination of tumor tissue; however, definitive diagnosis can be challenging in pleomorphic tumors that have morphological characteristics similar to other sarcomas, carcinomas, or round cell tumors. HS cells are large, discrete, mononuclear cells that display marked anisocytosis and anisokaryosis (Figure 32-27). Nuclei are round, oval, or reniform with prominent nucleoli and cytoplasm is moderate to abundant, lightly basophilic, and vacuolated. Mitotic figures are common and some tumor cells may display erythrophagocytosis or multinucleated giant cells.[52] Confirmation may be obtained using immunocytochemistry or immunohistochemistry on formalin fixed tissues using antibodies to CD18, the α_2 subunit of the major adhesion molecule family of leukocytes.[3] Macrophages and granulocytes express 10-fold more CD18 than lymphocytes, thus for differentiation purposes, lymphomas should express very low or undetectable levels.[1] If fresh or frozen tissue is available, further subclassification of the cell of origin can be performed using antibody staining for the CD11 α subunits.[3]

Due to the multifocal or disseminated nature of this disease, complete staging of patients is recommended. Complete blood count and biochemical screens are often abnormal. Anemia is common and usually regenerative when caused by erythrophagocytosis from neoplastic cells. Leukocytosis, thrombocytopenia,
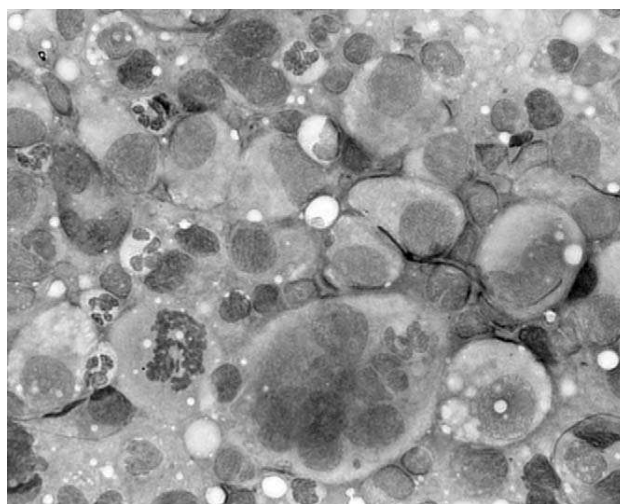
Figure 32-27

Cytology of HS. Neoplastic cells typically measure 15 to 30 microns and contain an ovoid nucleus with lightly clumped chromatin having zero to two variably sized indistinct nucleoli surrounded by a moderate volume of pale staining cytoplasm containing few small clear vacuoles. Few segmented neutrophils, a single giant multinucleated neoplastic cell, and an atypical mitotic figure are also present. *(Picture courtesy of Dr. Heather DeHeer.)*

increased liver enzymes and hypoalbuminemia are frequent findings, and the authors have occasionally noted hypercalcemia.[47] HS was the second most common cause of pancytopenia in dogs in a retrospective study of 51 dogs at a veterinary teaching hospital.[53] Thoracic radiography and abdominal ultrasonography commonly reveal abnormalities (Figure 32-28). Pulmonary involvement may appear as a diffuse interstitial infiltrate, patchy consolidated areas, or focal or multifocal mass lesions. Radiographic evidence of sternal, cranial mediastinal or tracheobronchial lymphadenopathy may also be noted. Hepatosplenomegaly, splenic or hepatic mottling, or masses in these organs are the most common abdominal ultrasonographic abnormalities.[54]

Bone marrow aspiration cytology may reveal tumor infiltrate, especially in patients with cytopenias. In addition, flow cytometry has been used to differentiate the etiology of hemophagocytosis in bone marrow samples containing over 5% macrophages and cytologic evidence of hemophagocytosis.[55] Results suggested that cellular distribution in scatter plots and number of histiocytes may help differentiate neoplastic from non-neoplastic causes of hemophagocytosis. Hyperferritinemia has been documented in dogs with HS and is theorized to be result of ferritin production by tumor cells.[56] In addition, attempts have been made to use serum ferritin levels to differentiate HS from other disease processes.[57]
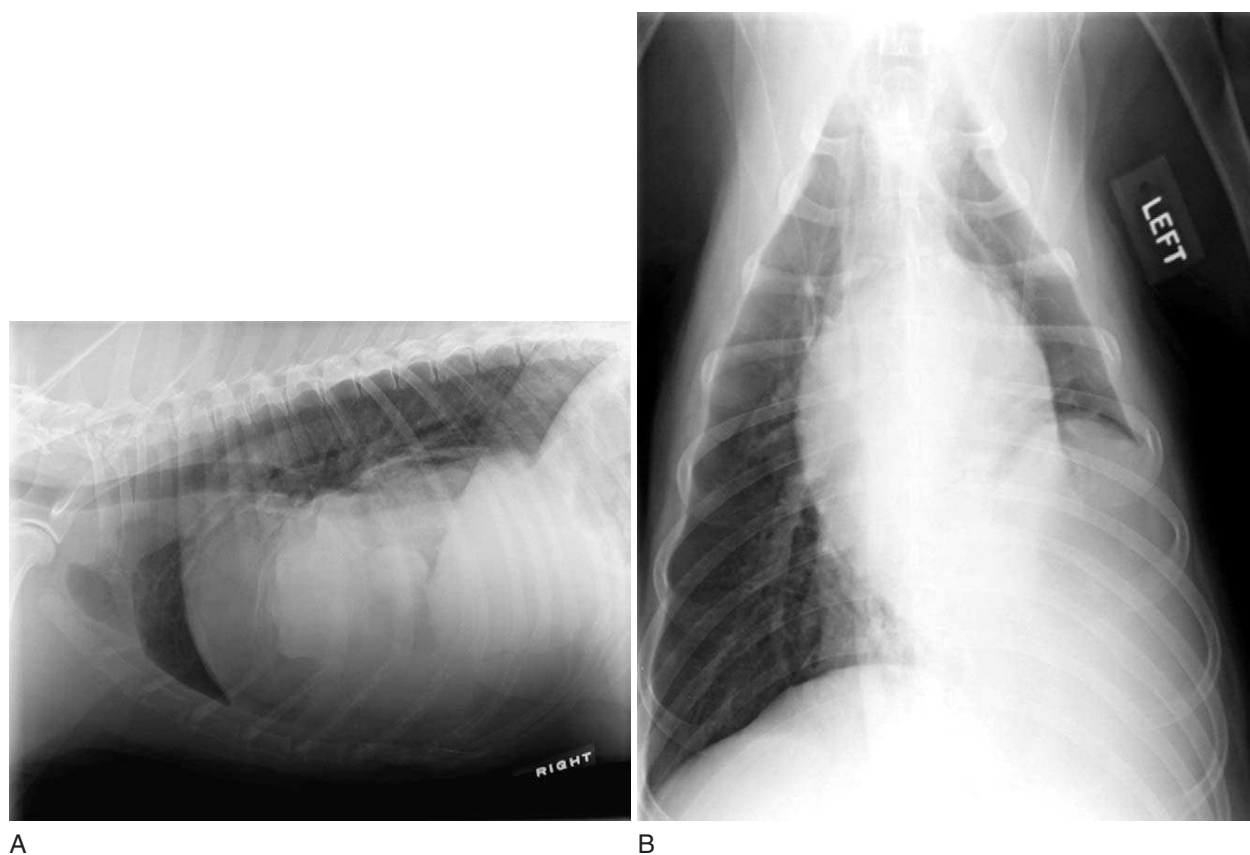
Figure 32-28

A, Right lateral radiographs of a 6-year-old FS German shepherd demonstrating a large, soft tissue mass and an adjacent yet separate smaller mass in the left caudal thorax. The mass displaces the caudal portion of the left lung bronchus and completely obscures the caudal bronchus. **B**, Ventrodorsal radiographs of the same patient demonstrating a soft tissue density in the left caudal thorax. Histopathology confirmed histiocytic sarcoma. *(Picture courtesy of Dr. LP de Lorimier; radiographic description courtesy of Dr. Anthony Fischetti.)*

Treatment and Prognosis

The clinical course of disseminated HS is rapid and usually fatal, whereas the localized form may be more amenable to therapy. Few reports documenting survival duration after surgical excision of localized histiocytic sarcoma exist. However, in a series of 18 synovial HS confirmed with CD18 staining, the overall median survival time (MST) was 3.6 months, the MST for dogs undergoing amputation (with or without chemotherapy) was 6 months, and the metastatic rate was 91%.[49]

Chemotherapy has been largely unstudied, although reports of responses to liposomal doxorubicin and paclitaxel chemotherapy exist.[58,59] In addition, a case report of a dog with cutaneous disseminated HS documented temporary remissions resulting from multiple protocols including cyclophosphamide, vincristine, prednisone, mitoxantrone, dacarbazine, and etoposide.[60] The authors have experience treating dogs with HS with CCNU, and a 46% response rate was noted in a multi-institutional study that included 56 dogs with advanced gross measurable disease. Median remission duration was 84 days and MST was 106 days. In this group, thrombocytopenia (less than 100,000/µl) and hypoalbuminemia were associated with a grave prognosis. Longer survivals were seen in dogs treated for microscopic disease or with multi-modality therapy.

Radiation therapy for HS has not been adequately reported; however, anecdotal responses have been described. Further study in this area is necessary. Durable clinical remissions and prolonged survival have been documented in four dogs with HS treated systemically with immunotherapy consisting of a human cytotoxic T-cell line (TALL-104);[61] however, this therapeutic modality is currently unavailable.

Feline Histiocytic Sarcoma

Histiocytic neoplasms are more rare in cats than in dogs. Most cats present with multifocal or disseminated

HS, and reported organs of involvement include the CNS, spleen, liver, lymph nodes, lung, mediastinum, kidney, bladder, and bone marrow.[50,62,63] Severe regenerative or nonregenerative anemia and thrombocytopenia are common findings, and bone marrow involvement is common in cats with three of three cats in a case series having positive bone marrow on postmortem evaluation.[63] An aggressive clinical course is typical, and results of treatment of HS in cats are not documented.

Comparative Features

Tumors of histiocytes and dendritic cells are rare in people and many purported cases have been reclassified as lymphomas based on immunohistochemistry. Lack of specificity of monoclonal antibodies such as CD11a-c and CD18 has been of concern in the differentiation of these cases, and cross reactivity with B and T-cell neoplasms has been documented. Many cases of HS and malignant histiocytosis in humans have been, therefore, reclassified as anaplastic large cell lymphomas, mostly of T-cell subtype.[64]

True HS in humans presents in the lymph nodes in one third of patients, in the skin in one third of patients, and in extranodal sites in the remaining patients. The intestinal tract is the most common extranodal site and hepatosplenomegaly is common. Some patients may also present with multiple anatomic sites of involvement, in which case the term *malignant histiocytosis* is applied and 70% of patients present with advanced stage (III or IV) disease.[64] HS in humans is an aggressive neoplasm that is often refractory to therapy and results in death in most patients. Langerhans cell sarcoma and interdigitating dendritic cell sarcomas are two other rare histiocytic neoplasms described in humans. Widespread disease in organs such as lymph nodes, intestine, liver, spleen, lung, and bone marrow is common and survival is usually poor.[64]

MALIGNANT FIBROUS HISTIOCYTOMA

The term *malignant fibrous histiocytoma (MFH)* refers to a group of tumors with histologic characteristics resembling both histiocytes and fibroblasts often displaying a storiform pattern with foam cells and multinucleated tumor giant cells.[65] In some cases, the term may be designated to poorly differentiated or pleomorphic forms of other soft tissue sarcomas.[66] With the development and increasing use of immunohistochemical techniques to further delineate tumor cell lineage, the malignant fibrous histiocytomas have become separated from tumors of true histiocytic origin based on positive vimentin, desmin, and S100 staining, but lack of CD18 and CD11 subunit staining. Some have suggested that this term be replaced by "undifferentiated sarcoma" or

"malignant spindle cell tumor" in the veterinary pathology literature.[31]

MFHs appear most commonly in the subcutis of both cats and dogs. They behave similarly to other soft tissue sarcomas (see Chapter 20) but appear in the dog to be more common in viscera such as the spleen. Storiform-pleomorphic, myxoid, inflammatory, angiomatous, and giant cell variants of malignant fibrous histiocytoma have been described.[67] They are locally invasive and can infiltrate deep into surrounding tissues making complete surgical excision difficult. Most subcutaneous tumors have a low metastatic rate, but high-grade forms may have greater metastatic potential. MFH occurs in cats as a type of injection site sarcoma and is commonly a giant cell variant.

In a histopathologic review of 98 cases of canine splenic fibrohistiocytic nodules by Spangler and Kass, a continuum of splenic nodular lesions from lymphoid nodular hyperplasia to pleomorphic sarcoma was described in which grade III fibrohistiocytic nodules were synonomous with MFH in this organ.[68] In this report, dogs with grade III nodules were more likely to die or be euthanized due to splenic disease than dogs with grade I or II nodules and survival probability at 1 year was significantly lower in dogs with grade III nodule. Splenic sarcomas including MFH have been reported to have a high metastatic rate and poor survival (4 months) after splenectomy, but the role of adjuvant chemotherapy in these cases has not been investigated.[69]

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